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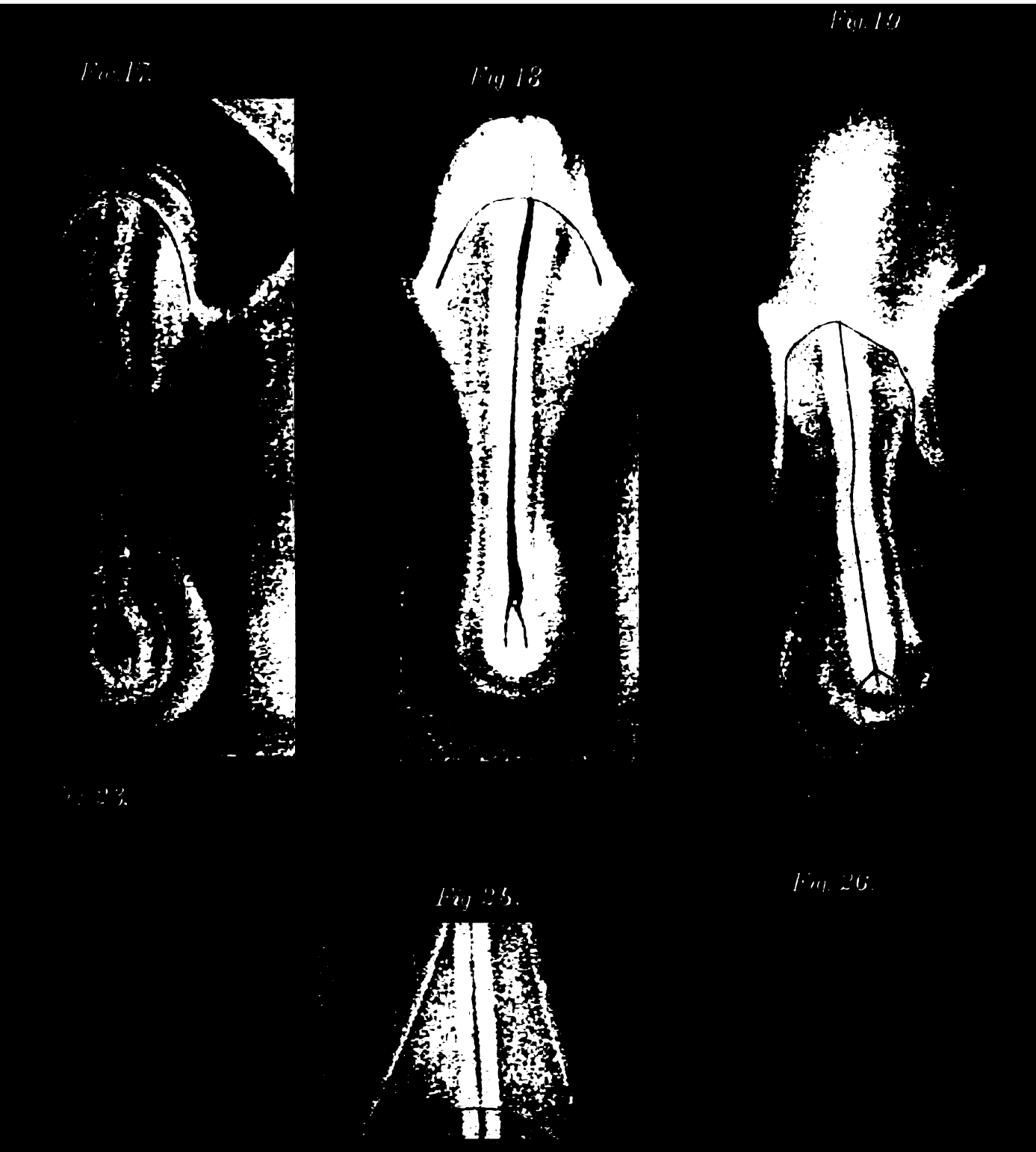

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東京帝國大學紀要

理科

第十冊

THE

JOURNAL

OF THE

COLLEGE OF SCIENCE,

IMPERIAL UNIVERSITY OF TOKYO,

JAPAN.

VOL. X.

東京帝國大學印行

PUBLISHED BY THE UNIVERSITY.

TŌKYŌ, JAPAN.

1896-98.

MEIJI XXIX-XXXI.

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PRINTED AT THE TŌKYŌ-INSATSU-KABUSHIKI-KWAISHA, TŌKYŌ

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帝國大學紀要

理科

第十册第一號

THE

JOURNAL

OF THE

COLLEGE OF SCIENCE,

IMPERIAL UNIVERSITY,

JAPAN.

VOL. X., PART I.

帝國大學印行

PUBLISHED BY THE UNIVERSITY.

TŌKYŌ, JAPAN.

1896.

MEIJI XXIX

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THE FOLLOWING CONTRIBUTION TO VERTEBRATE
EMBRYOLOGY IS DEDICATED TO THE MEMORY OF THE LATE
PROFESSOR FRANCIS MAITLAND BALFOUR
BY HIS DEVOTED PUPIL, THE AUTHOR.

On the Fate of the Blastopore, the Relations of the Primitive Streak, and the Formation of the Posterior End of the Embryo in Chelonia, together with Remarks on the Nature of Meroblastic Ova in Vertebrates.

(Contributions to the Embryology of Reptilia, V.)

By

K. Mitsukuri, *Ph. D., Rigakuhakushi,*

Professor of Zoölogy, College of Science, Imperial University, Tōkyō, Japan.

With Plates I.—XI.

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I. INTRODUCTORY.

In certain early stages of reptilian embryos, such as that given in Fig. 1., Pl. I., there is seen in the posterior part of the embryonic shield a mass enclosed within the limbs of the horse-shoe shaped blastopore. This mass exists in previous stages, but becomes conspicuous at this period, through being distinctly circumscribed in front and at the sides by the dorsal opening of the blastopore. A series of transverse sections through a *Chelonia* embryo of this stage is given in Series I, Pl. IV., and the section *f* in that series goes through the level of this mass. The epiblast, which consists, in more lateral parts of the section, of columnar cells, dips down at the lips of the blastopore into the mesoblast with which it fuses. Between the two lips of the blastopore

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thus bending downwards, a mass of cells without any regular arrangement rises upwards until it reaches the level of the general surface of the embryonic shield. This is the section of the mass above referred to. In the first of the present series of Contributions,* ISHIKAWA and I gave a minute description of this mass, as it occurs in *Trionyx*, and from its relations to the adjacent parts homologised it with the yolk-plug of the amphibian ovum. We made this comparison independently of any previous writer and, believed ourselves to be the first to point out the existence of a yolk-plug in a meroblastic amniote ovum. I have since found that previously to our publication DUVAL ('84) had discovered a similar structure in the nightingale and in the fowl (*loc. cit.* figs. 15 & 32), and had enunciated a hypothesis which, although not exactly like ours, is of similar tenor. If I understand him aright, he did not directly homologise the mass sticking out between the lips of the blastopore (his fig. 15, PP) with the yolk-plug of the Amphibia, but called certain cells, arisen by secondary segmentation and resting on the mass (his fig. 10, *n* & *g*), the *globules de Ecker* as he considered them homologous with the *bouchon de Ecker* or yolk-plug (*p. 68*).

Since our publication, VAN BENEDEN ('88) seems to have observed a similar structure in Mammalia and regarded it in the same light as ourselves. He says :—"Der Boden des (Chorda-) Kanals, ist mehrsichtig und ist nach hinten in Zusammenhang einerseits mit dem Hypoblast, anderseits mit einer Zellmasse, die vom Boden der Primitivrinne zwischen den Lippen derselben hinaufsteigt. Sie entspricht offenbar dem Dotterpfropf der Amphibien und ist ohne Zweifel mit dieser Formation homolog."

---

* See the list at the end of this article.

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HADDON in his *Embryology* ('87) copies DUVAL'S fig. 15 in his fig. 37, and calls the mass that sticks out between the blastopore lips a *yolk-plug*. He says :—" A comparison of a transverse section of an uncoalesced primitive streak of a nightingale (fig. 37*) with the almost completed blastopore of a frog (fig. 62, C & D†) will further tend to demonstrate the complete homology of the two stages" (*p. 43*). He refers to our observations in *Trionyx* in connection with this point.

HERTWIG is evidently of the same opinion. He says in his *Lehrbuch* ('93, *4te Aufl., p. 117*) :—" Lehrreiche Querschnitte durch die Primitivrinne** in den ersten Stadien ihrer Entwicklung sind in den Figuren 93‡ und 94‡ dargestellt. Die erste zeigt uns die beiden Urmundlippen (fig. 93 *ul*) durch einen kleinen Zwischenraum getrennt, in welchem sich von unten her ein kleiner Hügel (*dp*) von Dottermasse, die eine Anzahl Kerne (Merecyten) enthält, hineinschiebt, dem RUSCONIschen Dotterpfropf einer Amphibienlarve (fig. 88 *dp*) vergleichbar."

Of recent writers on reptilian development MEHNERT calls the cell-mass in question " Caudalknoten " ('91, *p. 422*). He says (*p. 423*) :—" Die Zellen des Caudalknotens quellen anfänglich aus dem offenen Prostoma heraus und zeigen auf Querschnitten eine völlige Uebereinstimmung mit denjenigen Bildern, welche von dem Dotterpfropfe der Amphibien schon seit langer Zeit bekannt sind. MITSUKURI und ISHIKAWA haben bei *Trionyx japonicus* eine mit dem Caudalknoten der *Emys* übereinstimmende Bildung ermittelt. Beide

---

* *I.e.*, DUVAL'S fig. 15, above referred to.

† C is a horizontal section through the blastopore showing the yolk-plug, etc.

** *I.e.*, of a chick-embryo.

‡ Both the figures are copied from DUVAL ('84). Fig. 93 is DUVAL'S fig. 32 (very similar to fig. 15 which Haddon borrowed) showing the yolk-plug. Fig. 94 is DUVAL'S fig. 31, showing the section of the primitive streak.

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Autoren beschreiben dieselbe als ein Derivat des hinter dem Blastoporus gelegenen Primitivstreifenblastemes und homologisiren sie mit dem Dotterpfropfe der Amphibien und sagen ausdrücklich: 'This area we consider to be the remnant of the yolk-plug of RUSCONI found in the Amphibian embryo.'" While MEHNERT thus recognises a complete agreement in the relations of these parts in Amphibia and Reptiles, he himself does not express a decided opinion on it. A page further on he says:—"Schon KUPFFER deutete den Randhügel* als ein Rudiment. Diese Anschauung is auch für *Emys* berechtigt, denn bei derselben ist der Caudalknoten zuerst relativ gross, um sich sodann später beträchtlich zu verkleinern. Seine Lagebeziehung zum Prostoma wegen kann man den Caudalknoten gewissermassen als ein peristomales Rudiment bezeichnen. In dem Caudalknoten scheint jedoch noch mehr zu stecken. Seine Verwachsung mit den hinteren Enden der Medullarwülste, seine Beziehungen zur Bildung der Schwanzspitze, das spätere Auftreten eines sich rückbildenden Schwanzknopfchens resp. Schwanzfadens spricht für eine tiefere morphologische Bedeutung. Das zeitliche Vorhandensein von so zahlreichen Nervenfasern wie in Sinnesorganen (MAX BRAUN) lässt an andere Beziehungen, etwa an ein rudimentäres Sinnesorgan denken, welches bei den jetzigen Vertebraten nur noch während des Embryonallebens zur Existenz gelangt." MEHNERT promises to describe further stages in the development of the "Caudalknoten" in a future paper—a promise which, so far as I know, he has not yet redeemed.

WILL ('92, '93, & '95a) calls the cell-mass in question the "Entodermpfropf" and considers it to be the homologue of the yolk-plug of the amphibian gastrula, as the following quotation will show

* KUPFFER homologises the "Caudalknoten" of *Coluber* with the "Randhügel" of the trout—which view is accepted by MEHNERT.

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('92, *p. 34*) :—" Wir sehen dass nur der vordere Abschnitt* sich an der Bildung der Einstülpung betheiligt, während der hintere* sich lediglich auf die Zellenwucherung beschränkt, im Niveau der Keimscheibe liegen bleibt resp. über das Niveau derselben vorragt und das Homologon des Dotterpfropfes der Amphibiengastrula darstellt, der bei den Amnioten passender als Entodermpfropf bezeichnet werden dürfte.

While all the authors above referred to hold views which are more or less like ours, there are others who have flatly opposed our interpretation.

ROBINSON and ASSHETON ('91) referring to our views as set forth in *Contribution I.* of the present series says :—* * * and in the tortoise, according to MITSUKURI and ISHIKAWA, the primitive streak consists, in the first instance, mainly of a mass of hypoblast or yolk, which they compare to the yolk-plug of Amphibians. To this, however, it cannot correspond, for we have already shown that the yolk-plug of *Rana* is a portion of the ventral wall of the archenteron, whilst the primitive streak is formed by the fusion of the lateral lips of a deficiency in the posterior wall of the same cavity."† (*p. 477*).

---

* *I.e.*, of the primitive plate. For the definition of this term, see further on.

† The above quotation shows that ROBINSON and ASSHETON have somewhat misunderstood our position, for we have never represented the mass in question to be a part of the primitive streak. That name we assigned, in our *Contrib. I.*, to a short axial strip, situated directly behind the yolk-plug where the epiblast is proliferating cells to the mesoblast (*Fig.* 8, *Contrib. I.*). This we regarded as caused by the coalescence of the posterior part of the blastopore lips, and not marked off from the yolk-plug, as it should be, because the posterior boundary of the yolk-plug remains rudimentary (*See Fig.* 6, *Contrib. I.*). This misunderstanding does not, however, affect the fact that ROBINSON and ASSHETON are opposed to our views on the yolk-plug homology. In a private letter to me, Dr. ROBINSON says: "* * * at present I am convinced that the mass of cells in the tortoise which you have figured and called the yolk-plug is a part of the primitive streak and is therefore not comparable with the yolk-plug of Amphibians * * ." In passing I may remark that what we called the primitive streak in our *Contrib. I.*, as explained above is the hindermost part of the primitive streak, if it is a part at all. The name, primitive streak should be given to an entirely different formation, as the facts brought out in the present Contribution will show.

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KEIBEL ('93) referring to the structure in Mammalia which VAN BENEDEN homologises with the yolk-plug in Amphibia :—" Der Dotterpfropf hat bei den Amphibien keine morphologische Bedeutung, er ist ein Entwicklungs hinderniss, er hat kein Funktion. Warum sollte gerade die Bildung so zäh festgehalten werden, während doch so vieles Andere dass von ungleich grosser Bedeutung ist, undeutlich wird und verschwindet ?" (*p. 105*). Further he says :—" Ganz räthselhaft erscheinen nur ferner die Beziehungen, die MEHNERT zwischen seinem Caudalknoten und dem Dotterpfropf und zwischen dem Primitivknoten und dem unteren von Dotterzellen gebildeten Eipol der Urodeln findet. Auch hier müsste der Dotterpfropf in der unteren Keimschicht in MEHNERT'S Paraderm, gesucht werden. Ganz unklar ist es auch, wie der Dotterpfropf mit dem hinteren Theil des Primitivstreifens der Säuger und deren Schwanzknopf resp. Schwanzfaden Beziehungen haben soll. Ein Verständniss für die vergleichend anatomischen Gesichtspunkte, welche MEHNERT hier geleitet haben, geht mir vollkommen ab." (*p. 105*).

In regard to the fate of the cell-mass in question, I wish to refer to two recent writers on the reptilian development viz :—MEHNERT, and WILL.

MEHNERT ('91) says :—" Bei noch älteren Embryonen (Fig. 17a, Fig. 18a*) hat sich das Caudalknötchen in eine caudalwärts gerichtete Spitze ausgezogen und kommt jetzt zwischen die hinteren Enden der beiden Medullarfalten zu liegen. Vergleiche mit früheren Stadien lehren dass der Caudalknoten sich unterdessen bedeutend verkleinert hat. Schliesslich verschmilzt der Caudalknoten seitlich mit den Endabschnitten der beiden Medullarfalten und bildet bei der Abschnürung des Schwanzes

* Substantially like Figs 18 and 9 of the present Contribution.

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das äusserste Ende desselben" (*p. 422*). He promises to give details in another paper.

WILL'S views on the changes occurring in the part where the cell-mass in question is situated are somewhat intricate, and the reader must refer to the original papers ('92, '93, '95) for a detailed description.* I can attempt to give here only the bare outlines.

Woodcut I.

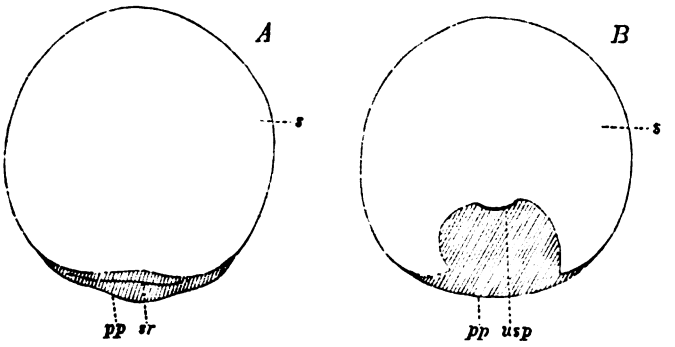

Diagram of the surface views of two embryos of *Cistudo lutaria* according to WILL. (*Zool. centralbl. I. p. 136*) *s*, embryonic shield; *pp*, primitive plate; *usp*, entrance to the archenteron; *sr*, sickle groove.

The primitive plate†, situated posteriorly to the embryonic shield, is at first in the shape of a sickle and the blastopore found in the middle of the plate is a transversely elongated slit (Woodcut I. *A*). Soon, however, the two horns of the sickle are grown over by the epiblast, by the process of the epibolic invagination, and there remains only the middle part of the primitive plate, which thus assumes a circular shape. By rapid cell-multiplication, the primitive plate now grows in length and becomes a median longitudinal streak (the *primitive streak*) which encroaches *forward* more and more on the embryonic shield (Woodcut I. *B*). A section through the primitive plate shows that, at its edges,

* WILL himself has recently given a summary of his own work in *Zool. Centralblatt, I. Nos. 4/5, 8, & 9.* For the point in question, see pp. 136-139.

† For the explanation of this term, see further on, or *Contrib. IV.*

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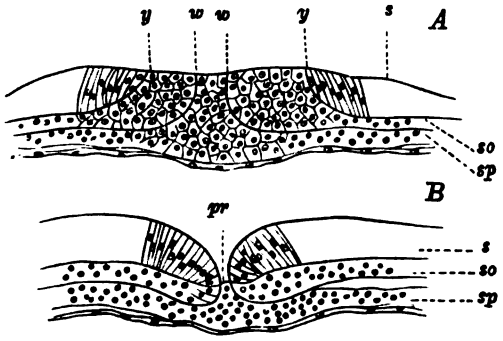
Woodcut II.

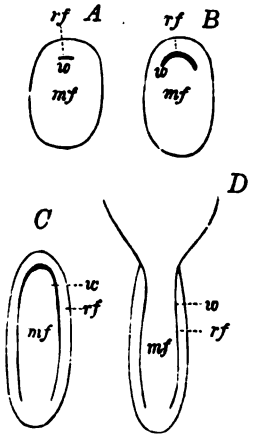


Sections through the primitive streak of a *Gecko* embryo of the VIIIth Stage, according to WILL (*Zool. Centralbl. I. p. 137*).

A 12 sections behind the anterior lip of the blastopore. *w-w*, central field; *y-w*, peripheral field.

B 2 sections behind the anterior lip of the blastopore. The peripheral field is fused with the ectoderm of the embryonic shield and is no longer distinct. The central field appears as the plug between the lips of the primitive grove, *pr*.

Woodcut III.



Diagrams showing the changes taking place in the primitive plate, according to WILL (*Zool. Centralbl. I. p. 138*). *w*, blastopore; *rf*, peripheral field; *mf*, central field.

the primitive plate is sharply marked off from the surrounding epiblast as shown at *y*, *y*, Woodcut II. *A*. The diagrammatic surface-view of the primitive plate would therefore be an area circumscribed by a distinct line all around, as in Woodcut III. *A*. The blastopore (Invaginationsöffnung), which is a narrow transverse slit in this figure, now begins to assume a crescentic shape, and its horns then gradually extend backwards over the entire primitive plate (Woodcut III. *B. C. D.*), *until they meet in the posterior median line.* Thus an elliptical central field (Mittelfeld) is circumscribed by a ring-like peripheral field (Randfeld), the line of demarkation between the two fields being formed by the blastopore and its posterior horn-like prolongation on each side (Woodcut III.). As the peripheral field is marked off from the central field, the

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former becomes continuous with the circumjacent epiblast, as shown in Woodcut II. *B.*, and the central field peeps out between the blastopore lips. This alone can therefore be called the yolk-plug. Now the lateral lips of the blastopore approach each other more and more in the median line. In WILL'S own words :—" Infolge der zunehmenden Annäherung der Urmundlippen wird nun die Mittelplatte (Fig. 11, II.*) mehr und mehr in die Tiefe gedrängt und zu einem Teil des splanchnischen Blattes, bis schliesslich bei vollkommener Verschmelzung der Lippen der Primitivrinne oder des Blastoporus zur Urmundnaht das Mittelfeld genau so überwachsen und invaginiert wird, wie bei den Amphibien der Dotterpfropf. Mit dem Zustandekommen der Urmundnaht ist demnach der Primitivstreif vollkommen von der Bildfläche verschwunden. (*Zool. Centralbl. I. p. 139*). Of the theoretical significance of these complicated processes, WILL says :—" In der Ausdehnung des Urmundspaltes nach hinten zu einem geschlossenen Ring beobachten wir demnach den gleichen Vorgang wie er bei den Amphibien stattfindet." (*loc. cit. p. 138*).

The foregoing brief survey of the literature bearing on the points at issue is enough to show that there is yet a great deal to be done, before any agreement of opinions can be arrived at in regard to the changes occurring at the posterior end of the embryonic shield in the blastoderm of the Amniota. And this is not to be wondered at, when we remember that in them the whole questions of the nature of the primitive streak, of the growth of the posterior end of the embryo, and of the origin of the allantois and of the proctodæum are involved.

After working out the process of gastrulation in Chelonia given

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* Copied in Woodcut II. B. of the present article.

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in *Contribution IV.* of the present series,* I decided to follow the fate of the cell-mass which ISHIKAWA and myself have homologised with the yolk-plug of Amphibia. The results obtained are beyond my expectations. I have been able to elucidate a series of facts, hitherto entirely unnoted, which seem to throw a great deal of light, not only on the cell-mass in question, but on the nature of the primitive streak and on the formation of the posterior end of the embryo. In consequence, I believe that the nature of the meroblastic amniote egg is made much clearer than heretofore, and that important conclusions can be drawn in regard to it.

My observations have been extended over three species of Chelonia, viz:—*Trionyx japonicus*, *Clemmys japonica*, and *Chelonia caouana*. The materials used were the embryos of *Chelonia* collected, as recounted in *Contrib. IV.*,* in the province of Tōtōmi, and those of *Trionyx* and *Clemmys* preserved from the more than abundant supply at the HATTORI turtle-farm in Fukagawa, Tōkyō. It is scarcely necessary for me to point out the great advantage of making a comparative study of nearly allied forms: observations on one species often throw a flood of light on points in other species which would otherwise remain inexplicable and obscure. In the present case, if I had had only the *Trionyx* embryos, I should probably not have succeeded, or at any rate should have had a great deal of difficulty in making out the course of development given in the following pages, while that species is indispensable in pointing out how the story so plain in the other two species has come to be abbreviated in higher forms.

Before proceeding further, it may perhaps be well to re-

* See the list at the end of the article.

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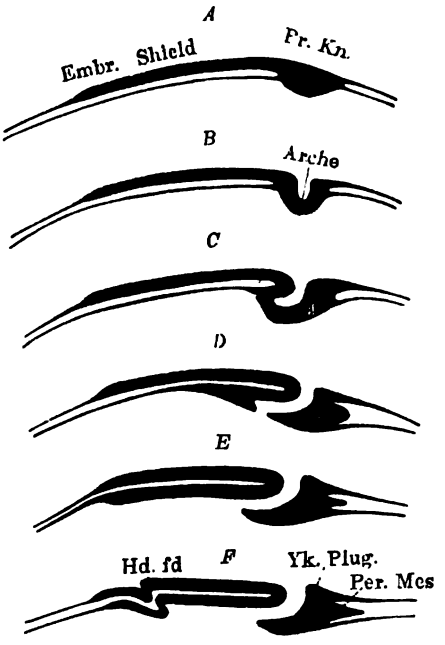
capitulate here briefly what has been given in the previous contributions, so far as is necessary to make what follows comprehensible. The facts are mainly contained in *Contribution IV.* When segmentation has gone on for some time, there is established in the blastoderm two layers: (*a*) the superficial epiblast composed of columnar cells, and (*b*) the lower layer consisting of irregular stellate cells and probably not forming a complete sheet (Woodcut IV. *A*). This separation into the two layers takes place in all parts of the blastoderm with the exception of a small area at the posterior end of the embryonic shield. Here not only is there no differentiation of layers, but a thick knob (*Pr. Kn.*) consisting of a network of cells is produced by the accession of cells from the subjacent bed of yolk. This is the *Primitive Knob* or *Primitive Plate.* The mass can not be said to belong to either of the two layers above named. According to my observations, the two layers pass gradually into the primitive knob, and nowhere can I discover such a sharp line of demarkation as is described by WILL (Woodcut II., *A*). Within the area of this knob, an invagination cavity is produced, which at first goes straight downwards, but very soon takes a forward horizontal course (Woodcut IV., *B*, *C*). This I take to be the *Archenteron* and its dorsal opening to be

Woodcut IV.



Diagrams showing the course of gastrulation in Chelonia.

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the *Blastopore*. Almost simultaneously with this act of invagination, or more probably *even before it*, the epiblast extends back to the anterior lip of the blastopore, so that from very early stages there is in front of the blastopore no area free from the epiblast. The cells of the primitive plate now begin to spread themselves over the ventral surface of the embryonic shield, mainly in the anterior direction, and *pari passu* with it, the archenteron extends itself forwards (Woodcut IV. *C*). The roof of the archenteron, which becomes continuous with the epiblast of the embryonic shield at the anterior lip of the blastopore, assumes a columnar arrangement, the process beginning at the posterior end and proceeding gradually forwards. Out of the median part of it is established the *chorda*-hypoblast, and from a certain stretch of columnar epithelium on each side of it is developed the gastral mesoblast (See *Contrib. III.* for details). The floor of the archenteron is divided into two parts:—(*a*) the posterior which proliferates the peristomal mesoblast, and (*b*) the anterior which, losing definiteness, is finally absorbed together with the whole thickness of the cell-network placed beneath it, thus putting the archenteron in communication with the large subgerminal fluid-cavity which has been developing in the yolk beneath the embryo (Woodcut IV. *D*). After this breaking through on the part of the archenteron, the cells derived from the primitive knob spread themselves gradually over the entire ventral face of the embryonic shield in the form of a trabecular network (Woodcut IV. *E*). This, however, soon becomes changed into a compact sheet of cells, the process beginning at the part that corresponds to the original roof of the invagination cavity and gradually spreading centrifugally. A median transverse fold arises soon at some distance behind the anterior edge of the embryonic shield (Woodcut IV. *F*, *Hd. fold*) and marks the head of the embryo, and the anterior end of the archenteron. The whole embryo

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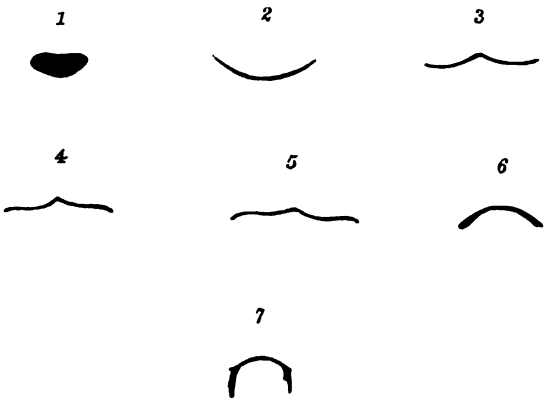
therefore arises within the area covered ventrally by the cells derived from the primitive knob.

It is necessary to call attention here to the change of shape which the blastopore undergoes in the course of the above events. Woodcut V gives outlines of the blastopore, as actually observed in one series of embryos of *Chelonia caouana*. As they were successively taken from the same deposit of eggs at certain intervals of time and as the changes of the shape are corroborated by other series of embryos similarly obtained, I think they may be assumed to be correct. In (*1*), the blastopore is a wide open cavity rather elongated in the transverse direction. In (*2*) it is a crescentic transverse slit, no longer gaping, with its concavity turned forwards. In (*3*), it is nearly straight across, in this case with a slight notch in the median line open backwards. In (*4*) and (*5*), the ends of the slit-like opening have been turned backwards so that the concavity now faces backwards. In (*6*), it is a deeper crescent, also with its concavity facing backwards. In (*7*), it has assumed a deep horse-shoe shape. In passing, I ought to mention that I have been able neither to discover the stage (reproduced in Woodcut I. *A*) given by WILL as occurring at the earliest period, presumably previously to (*1*) of Woodcut V., nor to make out any *forward* encroachment of

Woodcut V.



Diagrams showing changes of shape in the blastopore. The figures of entire embryos are given mostly in Contrib. IV. and that of (*7*) in Fig. *1* of this article.

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the primitive plate on the embryonic shield as given in Woodcut I. *B*.

II. NEW OBSERVATIONS.

In giving an account of the ontogenetic processes which I propose to describe in the present Contribution, it seems most convenient to go first over (*1*) the changes as they can be observed in surface-views of the embryos, and then (*2*) to describe the series of sections through the embryos, in order to explain more in detail the nature of the changes involved in these processes.

A. THE SURFACE CHANGES.

Before entering into the subject proper of this section I may be permitted to urge here the great necessity of studying *surface views* of embryos *with the utmost care* in investigations like the present. It seems to me that investigators, especially in vertebrate embryology have a tendency to make light of observations made of the surface views of embryos, and, before they have exhausted what can be learned from outside, have at once recourse to "microtomizing." I have never realised as in the present investigation the fact that a careful study of surface views not only saves a great deal of labour by giving a correct notion of the relations of the parts examined but *enables us to make out points which would almost certainly escape us if studied only or mainly in sections.* In fact, it would not be too much to say that but for an exhaustive study of the surface changes, this contribution would never have been written.

I found in the course of the present investigation that in most of those embryos in which the yolk-plug has already considerably shifted its position backwards (*see infra*), it could be made out only by turning

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them round and round in very bright diffused light or in not too strong direct sunlight until some one favourable position was secured. A paper *camera obscura* on the stage of the microscope cutting off light from undesirable quarters and a box of black wax in which white embryos stood out clearly, I found of great help in studying details. I also found that observations made on dark, grey skied days were not good for much, for I could invariably make out a great deal more from the same embryos on clear bright days, or even earlier in the afternoon than later. Examination by reflected light was far more useful than that by transmitted light. Staining was of little use, in the study of surface-views, for that process obscured many of the finer features. Great caution was necessary in arriving at negative conclusions : for it proved highly unsafe to conclude that a structure did not exist because it might not be visible in one or two looks. A long and careful study in the most favourable position and light was necessary before coming to a final decision. As a matter of fact, many of the embryos studied in the present investigation were some of those I used when working out the fœtal membranes of Chelonia (*Contrib. II*), but many of the features which I describe in the following pages entirely escaped me then and were therefore, not given in the figures accompanying *Contrib. II.*

In describing the surface-changes, I find it most convenient to treat the three species together in the earlier stages but to describe them separately in their later stages. For while the course of development is so much alike at first, *Trionyx* shows from a certain stage on considerable deviations from the other two, and these show some differences among themselves.

Earlier Stages

I start with the *Chelonia* embryo to which the blastopore given in

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Woodcut. V. 7 belongs. It is figured in its dorsal and ventral aspects in Figs. 1 and 1a, Pl. I. The head-fold has already sunk considerably so that in the ventral view it stands out prominently as a transverse ridge, marking the anterior end of the digestive canal. The anterior amniotic fold is distinctly seen as a horse-shoe shaped fold, although it leaves the head still exposed for the most part. The medullary groove is still very shallow, except in the posterior cephalic region where the medullary folds are raised higher than in front and back. In the posterior region of the embryonic shield there is in the median line a thick knob which stands out prominently on the ventral view. This is directly derived from the earlier primitive knob, although its nature has undergone a considerable modification by various processes accompanying gastrulation and by the proliferation of the peristomal mesoblast (*cf* Woodcut IV). Obliquely through this knob a canal passes from the dorsal to the ventral surface (Woodcut IV. F). This canal which I have called the blastoporic passage is the posterior part of the archenteron which has not had its floor absorbed. The dorsal opening of this canal, *i.e.*, the blastopore, is now horse-shoe shaped. The mass embraced within this horse-shoe is the structure which we have homologised with the yolk-plug in Amphibia. The ventral opening of the canal is rounded.

The medullary folds now arise. Three *Trionyx* embryos (Figs. 16, 17, 18 Pl. III.) are introduced to illustrate the relations of the medullary folds to the yolk-plug in the earlier phases of development. As the medullary folds (which, it will be remembered, arose first in the posterior cephalic region) proceed backwards and approach the region of the primitive knob, which may now more properly be called the terminal knob (Endwulst), they separate and enclose a wide space—the *sinus rhomboidalis* (Figs. 16 & 17). When they reach the yolk-plug, their posterior ends abut against the yolk-plug and

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embrace it, so to speak, between themselves (Fig. 17). This seems to raise the yolk-plug above the original level. It sticks out into the *sinus rhomboidalis* as a knob which is rounded in the case of Fig. 17. The dorsal opening of the blastoporic passage lies directly in front of it, and, as in Fig. 17, is often overhung by the anterior end of the yolk-plug and so is invisible in a dorsal surface-view. A narrow groove separates the yolk-plug on each side from the posterior end of the medullary fold of its own side and ought, I believe, to be looked on as corresponding to the backward-directed limbs of the horse-shoe shaped blastopore of the earlier stages.

The posterior portions of the medullary folds now press towards the median line and obliterate the *sinus rhomboidalis*, from the surface view at least. It exists within for some time longer. In coming together, the medullary folds compress laterally the yolk-plug, which thereby changes from its previous rounded shape to a triangular or wedge shape (Fig. 18). At this stage, one is sometimes able to see a delicate irregular white string or mass stretching in front of the anterior end of the yolk-plug and lying in the medullary canal. (There is a small detached mass in Fig. 18). This is perhaps the same thing as the "Achsenfaden" of DURSY,* and is probably the anterior end of the wedge-shaped yolk-plug detached and modified.

The course of the changes in *Chelonia* and *Clemmys* at these stages are very much like that just depicted for *Trionyx*. Fig. 2 of *Chelonia* and Fig. 9 of *Clemmys* are of embryos nearly corresponding to that of Fig. 18 of *Trionyx*.

The stages corresponding to Figs. 2, 9, and 18 have been noticed and figured many times by writers on reptilian development. The

* I unfortunately know DURSY's work only by a reference to it in GASSER's paper ('78).

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following are some of these instances:—STRAHL ('81), Taf. VI, Figs. 2 & 2a (like Fig. 2 of the present contribution); MITSUKURI & ISHIKAWA, Contrib. I, Fig. 4a; MEHNERT ('91), Tafel XVIII, Fig. 17a, 17b, 18a (*Cdl. Knt*); CLARKE ('91), Pl. XI, Figs. 27 & 28 (like Fig. 17 of the present contribution?). KUPFFER ('92) gives what appears to be a similar stage for the sparrow in his Taf. VIII, Fig. 6. It would no doubt be easy to add other instances, but these will be sufficient to show that such a stage has very frequently been noticed.

I have already quoted the views of two of the most recent writers on reptilian development as to the fate of this yolk-plug. According to MEHNERT, it becomes eventually fused with the posterior ends of the medullary folds and forms, when the tail is folded, its extreme posterior end. According to WILL, the blastopore lips gradually extend themselves backwards, until they meet behind, and entirely circumscribe, the cell-mass in question, which then becomes pushed in and disappears from the surface. All this takes place presumably before the upheaval of the medullary folds.

In *Contrib. IV*, I accepted WILL'S views as probably correct in the main, but I shall now proceed to show that the true course of development is different from either of the above views. In doing so, I find it most convenient, from the stage of Figs. 2, 9, and 18 onward, to separate *Chelonia* and *Clemmys* from *Trionyx*, as the former two display, in my opinion, the more primitive course of development, which is considerably obscured in *Trionyx*.

Later Stages.

I take up first

Chelonia caouana.

I would ask the reader to study carefully Figs. 2–8a which I

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have chosen for illustrating the course of the changes. Did it not increase figures to an inconvenient number, many other embryos might be represented, which would show phases of development similar to, or intermediate between, the stages given.

Fig. 2 has already been referred to, as showing the condition when the posterior portions of the medullary folds have pressed towards the median line and reduced the yolk-plug to a wedge-like shape, by embracing it between their posterior ends. Now follows a series of changes which, if expressed simply from the appearances observable on the surface and without any regard to the internal processes giving rise to them, may be objectively described as follows:—

The wedge-shaped yolk-plug, from this stage on, gradually shifts its position backwards. In doing so, it generally leaves in its track a shallow median groove which might be mistaken for the continuation of the medullary canal, although it can be easily distinguished from the latter by being narrower, shallower, and often more or less wavy.

I may state here by anticipation that I consider this groove as the *primitive groove*, and the region between the posterior portion of the medullary canal (with the blastoporic passage now changed into the neurenteric canal) and the backward-shifted yolk-plug as the region of *the primitive streak*. The cause of this backward shifting of the yolk-plug we must consider to be the extremely active growth and multiplication of the cells lying in the posterior portion of the embryo. These statements will be better understood, when the sections have been described. The backward progress of the yolk-plug at any given stage may roughly be estimated by its distance from the neurenteric canal and its position in regard to the "End-wulst" (terminal knob).

In Fig. 3 the medullary canal is already closed, except at its

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hinder end (the part delineated dark in the figure), and the neurenteric canal occupies about the middle two-thirds of this part, as can be readily determined by looking at the embryo by transmitted light. The yolk-plug, which can be easily told by its characteristic horse-shoe shaped outline, has therefore already considerably moved itself back, although it is still superposed, in the dorsal view, on the "Endwulst." This embryo is somewhat exceptional in that the groove between the posterior end of the medullary canal and the yolk-plug is ill defined, being shallower and wider than is usual, as a comparison with the succeeding figures will show.

In Fig. 4, the backward progress of the yolk-plug has gone one step further. As in Fig. 3, the posterior end of the medullary canal is still left open, as shown by the dark streak. This opening is somewhat irregular in shape, being wider in the middle and produced into narrower prolongations both in front and behind. The neurenteric canal is at the posterior end of the middle wider part. Now, the part shaded slightly lighter lying immediately around this wider part shows approximately the extent of the medullary canal as determined by transmitted light, so that all that part of the dark streak behind the middle wider part must be regarded as the groove stretching between the medullary canal and the yolk-plug. In this specimen, the anterior half of this groove is wider and appears as the direct posterior prolongation of that irregular opening which is still left in the posterior end of the medullary canal. At about the middle point of the groove, it becomes suddenly narrower and finer, and reaches the anterior edge of the yolk-plug. The latter, horse-shoe shaped as usual, appears now superposed on the edge of the "Endwulst."

In the embryo represented in its dorsal and ventral aspects in Figs. 5 (Pl. I.) and 5a (Pl. II.), the amniotic fold has extended

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nearly to the posterior end of the embryo and its free edge has the characteristic horse-shoe shape. The hinder part of this embryo is given on a larger scale in Fig. 5b. The posterior end of the medullary canal is still open so far as it is visible behind the free edge of the amniotic fold. The opening is in the figures at the anterior, wide, and darkest, part of the median streak seen behind the free edge of the amnion. The neurenteric canal also lies just under this opening, and marks the posterior end of the medullary canal. The light elliptical area around this end of the medullary canal is the "Endwulst." The yolk-plug, which has become somewhat pentagonal in shape, has now receded considerably behind the posterior edge of the "Endwulst" and is at the edge of the embryonic area (not the "*Stamm-zone*"). A delicate median groove extends between the posterior end of the medullary canal (delineated darker) and the yolk-plug, and is unusually distinct. In front this groove is straight and fine; behind it is a little broader and slightly wavy. The ventral opening of the neurenteric canal seen in the ventral view (Fig. 5a, Pl. II) is biscuit-shaped, being narrowest in the middle. This shape seems to be characteristic of *Chelonia caouana*.

In Fig. 6 is represented the hinder portion of another embryo, obtained from the same deposit of eggs at the same time as that given in Fig. 5. It also shows unusually clearly the relations of different structures in this part of the embryo at this stage. The medullary canal is entirely closed, with the exception of its most posterior portion. This opening, showing darker in the figure than the rest of the median streak, is slightly dumb-bell shaped, the medullary folds coming closer together in the middle of the opening than either in front or behind. This part also marks the position of the neurenteric canal. Behind this opening and in direct continuation of it, there stretches backwards in the median line a well-defined wide and shallow

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groove as far as the groove surrounding the yolk-plug. The latter structure is unusually distinct in this specimen and stands out prominently as a white area. It is almost geometrically hexagonal, not being marked by a well-defined groove only in its posterior boundary. Its position some distance behind the "Endwulst" and at the edge of the embryonic area is well shown in the figure, although there are differences in some particulars from what is seen in Fig. 5 or 5b.

There now ensues an important change, which Fig. 7 is introduced to illustrate, as well as another point. Here is seen one of those embryos in which a most careful search fails to reveal any trace of the yolk-plug. The primitive groove can also not be seen. There seems to be in different deposits of eggs great diversity in this respect. For instance, the embryos belonging to the series from which Figs. 5, 6, and 8 are taken show unusually clear figures of the yolk-plug, the primitive groove, etc., while in the series to which Fig. 7 belongs, the yolk-plug and the primitive groove are obscured. Histological details appear however to be essentially alike. The change which Fig. 7 is intended to illustrate lies in the *upheaval* of the tail-fold of the embryo. This is effected in the following way:— The "*Endwulst*," which can be recognised by the fact that it surrounds the posterior end of the medullary canal, becomes well marked in its posterior boundary by the gradual *rising upwards* of its own mass to produce the tail-knob. I may here anticipate the results of a study of the sections by stating that the tail-knob is not produced by the *tucking under* of the blastodermic surface, but by an actual *rising* or *uphaeval* of it at this part. The reasons for this conclusion will be given in the part dealing with the study of the sections. A similar figure (Fig. 25) in the *Trionyx* series will also illustrate this point. Referring again to Figs. 5 and 6, we may therefore conclude that the position to which the yolk-plug has retreated in these figures is

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considerably behind the tail-knob, inasmuch as the latter structure is produced by the rising upwards of the "*Endwulst.*" The groove extending between the posterior ends of the medullary canal and the yolk-plug, *i.e.*, the *primitive groove runs therefore, along the median line of the future tail-knob and, extending beyond it, reaches the yolk-plug.* The line marking the posterior boundary of the tail-knob crosses the primitive groove making right angles with it.

Figs. 8 (Pl. I.) and 8a (Pl. II.) represent the dorsal and ventral views of an embryo in which the tail swelling has become considerably elevated. The amnion has now not only covered the tail end of the embryo but extended far beyond it as the posterior tube of the amnion [described in detail in my *Contrib. II* and also, recently, by MEHNERT ('95)]. The lateral limbs of the posterior horse-shoe shaped opening of the amniotic tube are much longer in this than in *Trionyx* or *Clemmys*—a point characteristic of *Chelonia caouana.* With this extension backwards of the amnion, the tail-end of the embryo and the yolk-plug are necessarily covered by it but fortunately the yolk-plug is in this specimen seen unusually clearly through the amnion as a white mass, showing its position in the median line closely but distinctly behind the tail-swelling. In the ventral view also (Fig. 8a) the yolk-plug is seen as a median white structure behind the tail-swelling.

This is the last stage of *Chelonia caouana* in which I was able to detect the yolk-plug in the surface view, the complications of different structures in this region making surface observations impossible after this.

Clemmys japonica.

Let us turn next to *Clemmys japonica.* I only introduce here figures enough to establish the fact that an essentially similar course of events takes place in *Clemmys* as in *Chelonia.*

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Fig. 9 is the stage in which the medullary folds have reached posteriorly the yolk-plug and compress it laterally. The medullary canal is still open throughout. At the posterior cephalic region the medullary folds, which first arose here, are now pressed against each other, thus narrowing the medullary canal in that region in the surface view.

Fig. 10 is a sketch of an embryo, made long ago and produced in Fig. 2 of *Contrib. II.* I considered at the time that the horse-shoe shaped yolk-plug marked the posterior end of the medullary canal. A study of this figure, along with the series of sections prepared from the embryo represented in it, has made it evident that this is the stage in which the yolk-plug has already retreated a short distance behind its initial position. A slight enlargement of the medullary fissure a short distance in front of the yolk-plug, which I noted and sketched at the time, is undoubtedly the posterior end of the medullary canal and the line between that enlargement and the yolk-plug represents the primitive groove. This will become clear from the description of the sections prepared from the embryo (Series III. Pl. IV.).

Fig. 11 is the stage corresponding to Fig. 5 or 6 of *Chelonia*. The amnion leaves only the posterior part of the embryo exposed. As in *Chelonia*, the posterior end of the medullary folds is seen merging in the "Endwulst," which stands out as a circular white area. The medullary canal is completely closed, with the exception of a little stretch at its posterior end. At this place, there is a longitudinal opening, easily distinguishable by being wavy and a little wider than the rest of the median streak, This opening lies in a surface view at the anterior median radius of the "Endwulst." This also marks the position of the neurenteric canal in a surface view. From this opening, a delicate, and narrow groove—the primitive

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groove—goes backwards along the median line. It is much narrower than the posterior opening of the medullary canal. It is slightly wider and deeper in its hindmost part than in front. Just behind the spot where the primitive groove ends, a semilunar structure is faintly visible. This is the yolk-plug, differing somewhat in shape from that of *Chelonia*. In this specimen, I can not make out whether the primitive groove quite reaches the groove bounding the yolk-plug anteriorly. The "Endwulst," which is nearly circular in outline, is seen sending a small tongue backwards beneath the hindmost part of the primitive groove, which must be looked upon as the outwardly visible expression of the cells proliferated from this part of the primitive groove.

In Fig. 12, the medullary canal is entirely closed, but a slight widening behind is seen faintly through its roof which marks the spot where it was open, as in Fig. 11. and serves to indicate the position of the neurenteric canal. The primitive groove exists over only the posterior part of the "Endwulst", which sends out now a much larger and more distinct tongue backwards beneath the groove than in Fig. 11. The groove is wavy. Its hindmost part is slightly deeper, and in the surface view, darker than in front. In this specimen, there is no question whatever as to whether the primitive groove reaches the groove surrounding the yolk-plug. The latter structure is somewhat different in shape from that seen in Fig. 11, being top-shaped, with its pointed end turned forwards and insinuated into the primitive groove.

Fig. 13 is of about the same stage as Fig. 12. The medullary canal is completely closed, but the position of the neurenteric canal is still faintly visible from above as a slight enlargement of it. The tongue of the "Endwulst" projecting backwards is clear and decided. The primitive groove, which exists over only the posterior part of the

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"Endwulst," is straight in this specimen and distinctly reaches the groove surrounding the yolk-plug. This latter structure appears quite elongated and club-shaped. Careful examination shows that only the head of the club is surrounded by the groove and probably ought alone to be looked on as the yolk-plug. The part that corresponds to the stem of the club has only acquired the appearance of being continuation of the yolk-plug through being bounded on each side by a dark area which indicates an empty space within.

Fig. 14 represents an advanced stage in which the tail-swelling has already been considerably raised. There are two faint longitudinal streaks visible behind the tail-swelling and a third wedge-shaped one in between the two, some distance behind. The sections show that the primitive groove is continued some distance behind the tail-swelling, and the two parallel streaks are probably surface expressions of the lips of the primitive groove. Whether the third wedge-shaped streak represents the yolk-plug, I can not determine, as it is very faint and the sections unfortunately do not give a decided answer. This figure however is enough to show that the primitive groove is continued *behind* the tail-swelling.

Fig. 15 represents a much more advanced stage in which the amnion has already extended itself as a long posterior tube. The tail-swelling is also very much developed. Different from Fig. 8 of *Chelonia*, the yolk-plug is not visible behind the tail. There is, however, a long white streak present all along the median line of the floor of the posterior amniotic tube and extending even beyond its horse-shoe shaped postorior opening. This streak is often visible in *Clemmys* embryos, and I figured it in *Contrib. II.*, Pl. I., Figs. 5 & 6. It has also been noticed by MEHNERT in *Emys lutaria taurica*. ('95, Pl. X., Fig. 26). The discussion of its nature is postponed until we come to the description of the sections.

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The above account establishes conclusively, I think, that the changes occurring in the posterior part of the embryo run essentially the same course in *Clemmys* as in *Chelonia*.

Trionyx japonicus.

This species differs from the two foregoing species in that it does not show in any specimen the groove which connects the medullary canal with the yolk-plug. This I take to be evidence of its less primitive character. The histological details are however alike in all the essential characters, as will become evident when studying the sections.

Figs. 16, 17, and 18 have already been described, when showing how the posterior portions of the medullary folds are raised and how they come together and compress laterally the yolk-plug.

Fig. 19 is the stage next to Fig. 18. The medullary folds have met each other in the median line although not yet fused together in its posterior half. *The yolk-plug rests on the posterior ends of the folds*, and presents roughly speaking a pentagonal figure, distinct on all sides except behind, one of its angles coinciding with the line of meeting of the medullary folds. *Through* the yolk-plug the posterior end of that line of meeting can just be seen. A condition of the yolk-plug similar to that in this embryo, I was able to discover in several of the *Trionyx* embryos, but after diligent search nothing like it can be detected in any embryo of *Chelonia* or *Clemmys*. I am inclined to think that in these species, the yolk-plug gradually recedes after the stage resembling that in Fig. 18, without much changing its level in regard to the medullary folds, while in *Trionyx*, it is entirely lifted up above that level, attaining the condition given in Fig. 19 before it begins to move backwards. These circumstances have probably some relation to the fact that a primitive groove is

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left behind, so to speak, in the track of the yolk-plug in *Chelonia* and *Clemmys*, while it is absent in *Trionyx*, showing, as I have said, the less primitive character of the development of this genus.

In Fig. 20 the medullary canal is entirely closed and the yolk-plug with horse-shoe outline has receded somewhat, although in a surface-view it appears still superposed on the "Endwulst." The yolk-plug in this figure, as well as in some following, is represented a little too boldly. It is very hard to detect, and I discovered it only after searching for it long in not too strong direct sunlight. In a favourable light and position, however, it is distinct and unmistakable.

In Fig. 21 the yolk-plug is unnsually clear and has now receded some distance behind the "Endwulst," which appears white. There is no sign of the primitive groove.

Fig. 22 is very much like Fig. 21, but the shape of the yolk-plug is not as angular as in that figure.

In Fig. 23, the yolk-plug has receded about as far back as in Fig. 21, but here the backward directed tongue of the "Endwulst" is visible, and a faint narrow white streak can be recognised in the median line, passing under the yolk-plug, as a continuation of the tongue. This appearance is due to the cells proliferated along this line. Although the "Endwulst" in this figure thus appears different in shape from some which precede it, *e.g.*, that of Fig. 21, I believe it is not safe to conclude from this that this structure is different in reality, since the details visible in surface views depend so much on the conditions of preservation of the embryos and upon other secondary circumstances.

Fig. 24 (Pl. II.) is from an embryo which, in general appearance, is very much like that of Fig. 23. In it, however, I was unable to detect any sign of the yolk-plug. Just where the yolk-plug ought

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to be present, there is visible instead a median white streak, bounded on either side by a dark streak and on the right, a second fainter white one. It appears therefore, as though there were two distinct ridges here, but I believe that the right hand light streak is due to some secondary circumstance, and that the one in the median line alone indicates proliferation of cells in the epiblast and is comparable to the streak visible below the yolk-plug in Fig. 23.

Fig. 25 is important as showing how the tail-swelling arises. In this embryo, three concentric zones are visible in the part of the embryo exposed behind the posterior horse-shoe shaped edge of the amnion. These are simply the surface expressions of various thicknesses of tissues in different parts. The yolk-plug and a median streak under it are seen faintly on the outermost of these zones. The line that marks the outermost from the middle zone is somewhat faint. The innermost zone, however, is sharply marked off from the middle one and is to be looked on as showing the extent of the "End-wulst." It is in fact this zone which is now rising up as the tail-swelling, as will become evident from the sections. Here, again, it is evident that the yolk-plug lies clearly behind the tail-swelling. Fig. 25a (Pl. II.) is the ventral view of the same embryo, and shows well the ventral opening of the neurenteric canal.

Fig. 26 is the last stage in which I was able to detect the yolk-plug in a surface-view. The tail-swelling is now distinctly bounded by a deep groove all around. At the posterior edge of this groove, partly covered by the amniotic fold is the yolk-plug with its characteristic shape in the median line. Fig. 26a (Pl. II.) is the ventral view of the posterior portion of the same embryo. The semilunar notch behind the ventral opening of the neurenteric canal is the commencing allantois.

After this stage, the part where the yolk-plug ought to be visible,

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is covered over by various structures which render it no longer possible to be seen in a surface-view, even if present.

Summary.

The changes which I have described as observable in the surface-views of the embryos of the three species may be summed up as follows:—

The mass of cells which sticks out between the limbs of the horse-shoe shaped blastopore—i.e., the yolk-plug—is embraced between the posterior ends of the medullary folds, when these are formed, and is lifted and compressed laterally by their coming together.

It then apparently shifts its position backwards.

In Chelonia and Clemmys, the yolk-plug in thus moving backwards, leaves, so to speak, in its track a groove which therefore necessarily joins the hindmost portion of the medullary canal with the groove surrounding the yolk-plug. This is the primitive groove.

In Trionyx, such a groove is not visible on the surface.

The "Endwulst" rises up as the tail of the embryo, and the line of upheaval crosses the primitive groove (when such is present) at right angles and midway between the initial position of the yolk-plug and its final backward position.

The yolk-plug therefore is not included in the tail and lies some distance behind it.

B. THE SECTIONS.

In giving an account of the internal developmental processes of which the changes described in the preceding section may be said to be the surface expressions, the most logical way would be to give parallel series of sections of different stages in each of the three species that formed the objects of my research. Investigators of similar

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subjects will find it easy to understand that many little difficulties, however, stand in the way of realising such a course, and that the labour necessary in overcoming such difficulties is not at all commensurate with the advantages to be derived from it. Moreover, such a proceeding would increase the number of figures and plates to an inconvenient degree. I have, therefore, thought it best to present here series of sections, selected from the three species, in such a way that where the course of development is practically the same in two or all of the three species, a series from one species may do office for the other one or two. Series of sections are therefore given, mainly in the order of their age, regardless of the species to which each may belong Where there is a striking divergence between any of the three, that fact will be mentioned and as far as possible illustrated with a series of sections. The reader is for this reason requested to note specially to which species each of the series given in the following pages belongs.

The numerals placed between every two sections give the number of sections that intervene between those two. Again, when a section is stated to be so many sections behind another it must be understood that that section is excluded, and that counting begins from the section next to it and includes the given section behind. Karyokinetic figures are filled in in some of the series, and omitted from others, so that their absence in the drawing must not be taken as their absence in the sections themselves.

I confess, it is with some hesitation that I have decided to present the description of the series in the shape given in the following pages, for to read through a mere dry description of sections, even if accompanied by figures, is in my opinion one of the most tiresome things, and I am afraid I may be taxing unduly the patience of the reader. When, however, I consider that if he were present personally before me and I wished to convince

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him of what I wish to do in writing this paper, the best course open to me would be to make him look through my sections and to explain to him the nature of each series, (in addition to looking over the embryos *in toto*), I feel myself obliged to adopt, in writing, a course as similar to that as I can make it, and to pick out the series of sections that are important and to describe them. I, however, intend to be as brief as possible and to touch only on the salient features.

Series I.

(Pl. IV.)

Chelonia caouana.

An embryo, with no mesoblastic somites; taken out 9½ days after deposition ; surface views given in Figs. 1 & 1a, Pl. I.* (B 11 b).

In this series is given a number of transverse sections from the posterior portion of the *Chelonia* embryo given in Figs. 1 & 1a.

a is from the hinder part of the mid-dorsal region. The chorda in this region is distinctly formed, and is independent of all the surrounding parts: the mesoblast, the hypoblast, and the epiblast.

b is the eighth section behind *a*. The chorda is in the process of formation but is as yet in connection with the mesoblast and the gut-hypoblast.

c is the sixth section behind *b*, and is slightly in front of the ventral opening of the blastoporic passage. The chorda, as such, is no longer recognisable, and the *chorda*-hypoblast, the gastral mesoblast, and the gut-hypoblast are all in connection with one another.

* The age of the embryos given in days does not at all indicate the stage of development to which they have attained, as this depends on the temperature of the atmosphere and other external conditions.

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d is the fourth section behind *c*, and is just at the ventral opening of the blastoporic passage. As the section is somewhat oblique, the passage is nearly open below on the left side. The dorsal and lateral walls of the passage show an arrangement of cells like columnar epithelium, while the bottom is formed of a mass without any regular arrangement. The epiblast is fused with the *chorda*-hypoblast, as we are already in the region where the epiblast is reflected and becomes continuous with the hypoblast at the dorsal lip of the blastopore.

e is the second section behind *d*, and is at the dorsal opening of the blastoporic passage. The epiblast is reflected on each side at the lips of the blastopore and is proliferating the peristomal mesoblast. The bottom of the passage is formed of a mass of cells without any regular arrangement.

f is two sections behind *e*. The mass of cells which formed in *d* and *e* the bottom of the blastoporic passage stands out in this section as a prominent body between the lateral lips of the blastopore which are proliferating the peristomal mesoblast as before. This mass is the so-called *yolk-plug* and shows no regular arrangement of cells. The gut-hypoblast, which was fused in *d* and *e* in the median line with the mass above, is in this section clearly independent. In the preceding section the yolk-plug is distinct, but smaller.

g is four sections behind *f*. The groove on each side between the lateral blastoporic lip and the yolk-plug has been getting shallower with each section behind *f*, cells on the surface are arranged like columnar epithelium and although as yet continuous with the peristomal mesoblast below, we must regard this region as that of transition to the epiblast. There is even an indication of the separation of the upper layer from the subjacent mesoblost for a very short stretch in the median line. Compare Woodcut VI. further on

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for this point.

In the next section, the epiblast is separate from the subjacent mesoblast in the middle of the yolk-plug. In three or four sections more, all the three layers are independent of one another.

Woodcut IV., *F'* (p. 12) may be taken as the diagrammatic longitudinal section of this stage.

This series shows that in the embryo given in Figs. 1 & 1a, there is in the posterior part a blastoporic passage which takes an oblique forward course from its dorsal to its ventral surface. The dorsal opening of this passage is horse-shoe shaped (Fig. 1) and the outer edges of this opening are proliferating the peristomal mesoblast in all its parts, excepting the anterior lip where the epiblast is reflected and continuous with the chorda-hypoblast. The mass of irregularly arranged cells, which forms the floor of the blastoporic passage and is also continuous with the peristomal mesoblast, sticks out to the level of the dorsal surface between the horse-shoe shaped lips of the blastopore, and is bounded anteriorly and laterally by them. This is the mass which I have called the *yolk-plug*. Posteriorly I have not seen this mass bounded by a groove in any specimen. But as the yolk-plug passes posteriorly gradually into the epiblast, and as the region of this transition is continuous with the peristomal mesoblast, (Woodcut IV. *F*), we may suppose the blastopore lip to be present here in a rudimentary form. I wish also to call attention to the fact that all the three layers are fused on the floor or posterior wall of the blastoporic passage (*Cf.* Woodcut IV. *F*).

As Section *f* of this series shows, there is no epiblast on the dorsal surface of the yolk-plug. I have reconstructed from the series a surface view of the posterior portion of the embryonic shield for the purpose of ascertaining the extent of the area thus free from the

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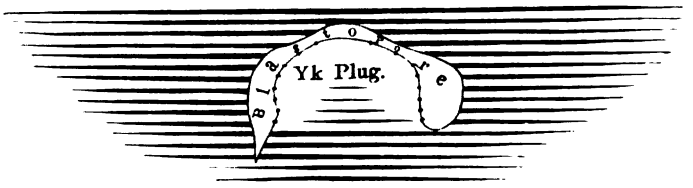
Woodcut VI.



Reconstructed surface-view of the posterior portion of the embryonic shield.
[Enlargement in length is slightly less than enlargement in breadth].

epiblast. Woodcut VI. is such a reconstruction. The heavy dark lines are intended to show the epiblast. It will be seen that the mass embraced within the horse-shoe shaped blastopore is entirely free from the epiblast. As we go backwards, the surface layer gradually acquires an arrangement like columnar epithelium (see *g*) and the thus arranged layer little by little separates itself from the subjacent mesoblast, first in the median line and then by degrees laterally until there is an independent epiblast layer over the entire breadth. Short transverse lines in the posterior part of the yolk-plug in Woodcut VI. indicate the extent of the separation of the topmost layer from the mesoblast below. It is natural that the fusion of the upper layer with the mesoblast goes backwards furthest along the groove or lips of the blastopore, as the mesoblast is being proliferated here.

Stages similar to, or preceding, this in *Clemmys*, *Trionyx*, and *Chelonia* have already been described in *Contributions I, III, and IV.* In them, the mesoblast formation will also be found described.

Series II.

(Pl. IV.)

Trionyx japonicus.

An embryo with 2-3 mesoblastic somites; taken out 4 days after deposition; surface view given in Fig. 18, Pl. III. (136β).

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In this is given a series of transverse sections through the region of the yolk-plug in the *Trionyx* embryo represented in Fig. 18 (Pl. III.).

a has the medullary canal wider than in the part anterior to it, showing that although the *sinus rhomboidalis* has been obliterated from the surface-view (compare Fig. 18 with Fig. 17) it still exists within. The medullary chord and the *chorda*-hypoblast show already a tendency to merge into each other. The yolk-plug appears in the medullary canal as a mass of cells resembling in shape the odontoid process of the *axis*. A comparison with Series I, *f*, shows that the yolk-plug has greatly diminished in size. The fact that the part of it lying within the medullary cavity is larger and more rounded than the part embraced between the edges of the medullary folds shows that the approachment of the medullary folds towards the median line has compressed the yolk-plug laterally. Note that the karyokinetic figures are numerous in the walls of the medullary-, and chorda-, canals in this and succeeding sections, showing that this is the seat of active growth.

Anterior to this section, there are two in which the front termination of the yolk-plug is seen as a mass much smaller than that in *a*.

b is the section next behind *a*. The medullary-, and the *chorda*-, canals approach each other and the fusion of their wall is greater.

c is next behind *b*. Here, we are in the region of the neurenteric canal, *i.e.*, the medullary canal has united with the *chorda*-canal. Owing to the slight obliquity of the plane of the section, there is on the left side (right, in the figure) of the neurenteric canal a ledge of cells projecting transversely into the middle of the canal. Comparing this section with Series I. *f*, we perceive that the upper surface of the yolk-plug was in the latter section at the

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level of this ledge, and that the uprising of the medullary folds has therefore carried the yolk-plug upwards along with them. This perhaps accounts for the great diminution in the breadth of the yolk-plug, since we must consider the same mass of cells spread over a greater extent.

d is two sections behind *c*. The posterior wall of the neurenteric canal (earlier the floor of the blastoporic passage) which is formed of a mass of cells without any regular arrangement is nearly reached, and a part of it is seen on the right (apparent left) side standing up from the bottom of the canal. The yolk-plug is in contact, but is not yet fused, with it. The wall of the medullary canal is now inseparably fused with the mesoblast along the larger part of its outer side.

e is the section next behind *d*. The yolk-plug has now united with the posterior wall of the neurenteric canal and is seen as a tall mass standing up from the bottom of the canal. Its most superficial part is somewhat marked as a cap sitting on the underlying part. This probably is the first indication of the epiblast which, as a comparison with Woodcut VI. shows, encroaches on the dorsal surface of the yolk-plug before the posterior points of the lateral limbs of the horse-shoe shaped blastopore are reached.

f is the next section behind *e*. The groove on each side of the yolk-plug is becoming shallower, as we are nearing the ends of the lateral limbs of the horse-shoe shaped blastopore.

g is two sections behind *f*. The epiblast of the right (apparent left) side has become continuous with the epiblast over the dorsal surface of the yolk-plug. In the left (apparent right), there is still a groove present. The columnar-epithelium-like arrangement of cells marking the posterior ends of the medullary chord which has been getting less and less in the last few sections has almost

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entirely disappeared, excepting a little stretch on the left.

h is the section next to *g*. The epiblast now extends over the entire breadth, but there is still a mass of cells in the median line, immediately below the epiblast, which is connected with the superjacent epiblast and with the subjacent mesoblast. This mass, which I have darkened in the figure from conventionality, is in fact lighter and quite noticeable. It gradually decreases until in the fifth sections behind *h*, the epiblast is distinctly separate throughout from the mesoblast below, and thus the three layers become all independent.

The obliquity of the blastoporic passage, which has now become the neurenteric canal, is much less in this series than in Series I.

Fig. 9 (Pl. II.) of *Clemmys* and Fig. 2 (Pl. I.) of *Chelonia* are the stages corresponding to this in *Trionyx*. The main points of these embryos are like those of this series, although different in various details, since the idiosyncrasies of individual embryos not only in different species but within the same species are as a general thing very great.

Series III.

(Pl. IV.)

Clemmys japonica.

An embryo with 5 mesoblastic somites; taken out 4 1/2 days after deposition; represented in Fig. 10, Pl. II. (=Contrib. II., Plate I., Figs. 2 and 2a). (XXXXIX).

In this is given a series of transverse sections through the posterior part of the *Clemmys* embryo represented in Fig. 10. The yolk-plug has already receded a little from its original position.

a is from the hinder portion of the mid-dorsal region. The medullary canal is still open.

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b is nine sections behind *a*, and is the second in which the medullary chord shows signs of fusion with the notochord. The mesoblast and hypoblast are independent.

c is five sections behind *b*. The notochord can no longer be recognised as such. The *chorda*-hypoblast is fused above with the medullary chord, laterally with the mesoblast, and is continuous below with the gut hypoblast.

d is four sections further back than *c* and is the first in which the neurenteric canal is open below. The canal is now almost vertical. The medullary chord is now fused with the mesoblast along the entire height of its sides. The epiblast, the medullary chord, the mesoblast, the *chorda*-hypoblast and the gut-hypoblast are all united in this section.

e is three sections behind *d* and is the first in which the neurenteric canal is closed ventrally. From the floor of the canal stands up a small club-shaped mass. Its continuation is also seen in the next section *f*. This is probably the same thing as what is called the "Achsenfaden" by DURSY. It seems most reasonable to suppose that such a yolk-plug as that described in Series II (*c*, *d*, *e*,) has left a few cells here in moving backwards.

f is the section next behind *e*. There we reach the posterior wall of the neurenteric canal which can be recognised by the columnar-epithelium arrangement.

g is two sections behind *f*. Here we have already left the region of the medullary groove and we see the section of the groove which appeared like the posterior prolongation of that canal in the surface view—we called it the *primitive groove*. Note that the epiblast which was continuous with the wall of the medullary canal in all the preceding sections bends down in this section on each side of the aforesaid groove to become directly continuous with the peristomal mesoblast. The cells arranged to form the posterior wall of the neurenteric canal are still recognisable.

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h is two sections behind *g*. Here we see the last vestiges of the posterior wall of the medullary canal. Otherwise it is very much like *g*.

i is two sections behind *h*. There is no longer any trace of the posterior wall of the neurenteric canal. The groove, etc., are very much as in *g* and *h*.

In the next section behind *i*, there is a sudden falling in the height of the section. We must consider the "End-wulst" as extending from *c* backwards to that section.

j is three sections behind *i*. The groove is wider than in front, and in it, there is a small projection from the bottom—the anterior end of the yolk-plug.

k is five sections behind *j*. The yolk-plug has gradually been enlarging from *j* backwards and in this section we see a distinct yolk-plug separated on each side by a groove from the epiblast which dips down to fuse with the mass below. This is substantially like Series I, *f*, and Series II, *f*, and shows conclusively that compared with those series, *the yolk-plug has shifted its position to one some distance behind the posterior end of the medullary canal, the intervening space between its original and present position being traversed in the median line by a groove on each side of which the epiblast is proliferating cells to the subjacent mesoblast.*

l is four sections behind *k*. The yolk-plug is lower and the groove on each side of it is shallower.

m is six sections further back. The epiblast now stretches over the entire upper surface, but the backward continuation of the yolk-plug is still marked by a slight elevation (included between two stars in the figure) and is fused with the mesoblast at one point. After this section, the layers become independent.

It is hardly necessary to point out that of the above sections, *g*, *h*, and *i*, *are very much like sections through the primitive streak of the vertebrates in general. In fact I take the region represented by these three*

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sections to be the primitive streak of this embryo and the groove that appears like the posterior prolongation of the medullary canal to be the primitive groove. The primitive streak and groove therefore extend from the neurenteric canal in front to the yolk-plug behind. At the posterior end, the groove is pushed apart, as it were, to allow the yolk-plug to peep out on the surface. The mass formed by proliferation from the primitive streak is mostly taken into the thick knob, the "*Endwulst.*"

In this embryo, the yolk-plug extends over nearly sixteen sections. It seems to be longer in *Clemmys* than in the other two species.

Series IV.

(Pl. IV.)

Trionyx japonicus.

An embryo with 4 mesoblastic somites; taken out 5 1/2 days after deposition; represented in Fig. 19 (Pl. III.) and also in Contrib. II. Pl. III. Figs. 17 & 17a. (128a).

In describing the surface changes, it was mentioned that *Trionyx* behaves somewhat differently from *Clemmys* and *Chelonia* in the backward movement of the yolk-plug, and that after passing through the stage like Fig. 18 it becomes like Fig. 19, and the yolk-plug rests flat on the posterior end of the medullary chord. This series is composed of the selected transverse sections from the embryo represented in Fig. 19.

a is from the hinder portion of the mid-dorsal region. The medullary canal is still open and its wall continuous with the epiblast.

b is seven sections behind *a*. The *chorda dorsalis* is continu-

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ous with the medullary chord above, with the mesoblast on the right (apparent left) side, and with the gut-hypoblast below.

c is four sections behind *b* and is the first in which the neurenteric canal is open below. On the dorsal surface of this canal is seen the section of the anterior termination of the yolk-plug, which is independent of any adjacent structure. The section is slightly oblique: hence the asymmetry of the yolk-plug.

d is five sections behind *c*. The neurenteric canal is closing below. On the median line above, is a funnel-shaped depression and in it rests a triangular mass—the section of the yolk-plug. As karyokinetic figures seemed especially numerous in this section, their places have been indicated.

e is two sections behind *d*. We are entirely out of the region of the medullary chord. There is a median groove that goes from above deep into the "Endwulst." We must regard this as the rudimentary primitive groove. The section of the yolk-plug rests like a wedge on the funnel-shaped upper part of this groove.

f is two sections behind *e*. There is no deep median groove in the "Endwulst," but the epiblast is still continuous with the mass below in the median line. The section of the yolk-plug rests in a depression of the surface.

g is the second section behind *f*. The yolk-plug is continuous on the right (the left of the figure) with the epiblast, while still marked by a groove on the left. The epiblast is continuous with the mesoblast below in the median line.

h is five sections behind *g*. The yolk-plug does not stand out prominently but its extent (comprised between two stars in the figure) is easily recognised by the peculiar arrangement of cells unlike that of the epiblast—the nuclei being packed without order. The lower face

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of the epiblastic layer shows in this section a median indentation which goes upwards and the mass below seems to become *directly* continuous with the yolk-plug —an arrangement different from what obtains in *g*. It would be reasonable to suppose that the *primitive streak* ends posteriorly at the point where the arrangement as in *g* (*i.e.*, the fusion of the *epiblast proper* with the mass below) ceases and that the arrangement as in *h* represents the yolk-plug standing up from below like Series I, *f* or Series III *k*. The limit is very difficult to define, as we must consider all this as existing in a rudimentary condition, but I should say it is present about two sections in front of *h*.

i is the third section behind *h*. The epiblast is now entirely independent of the mesoblast, but there is still a faint median elevation (indicated by two stars) which is the continuation of the yolk-plug. The mesoblast also sends up a median projection towards the epiblast.

I think, it is easy to see from this series that the course of events is very much less primitive in *Trionyx* than in the other two species.

Series V.

(Pl. V.)

Chelonia caouana.

An embryo with 9 mesoblastic somites; taken out 9 1/2 days after deposition, represented in Fig. 5 (Pl. I.), 5a (Pl. II.) & 5b (Pl. I.)

(B11 ∧)

This is much older than any of the series above described and is one of the most important. The yolk-plug has now receded a considerable distance backwards.

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a is from the hinder portion of the dorsal region. The medullary canal is closed.

b is ten sections behind *a*. In passing from *a* to *b*, the notochord and the medullary chord have gradually been fusing with each other, until in *b* the former looks merely like the thick ventral wall of the latter, although the irregular arrangement of cells reveals its nature.

c is four sections behind *b*. The noto-medullary chord is now connected with the gut-hypoblast and on the right (apparent left) side, with the mesoblast for a short extent.

d is two sections behind *c*. The section in front of this and therefore next to *c* is the first in which the neurenteric canal is open below. The wall of the canal is continuous on either side with the gut-hypoblast, and with the mesoblast for a short stretch directly above its junction with the gut-hypoblast. The wall of the neurenteric canal is also just fusing with the epiblast above.

e is the section next behind *d*. It is like *d* in every respect except one, viz.: the epiblast is now distinctly continuous on each side with that half of the wall of the neurenteric canal which is on its own side, and the canal is thus open above. This corresponds of course to that part in the surface view (Figs. 5 & 5b) where the medullary canal is open above.

f is two sections behind *e* and is from about the middle of the ventral opening of the neurenteric canal. It is in almost every respect like *e*, but is introduced to show that the breadth of the neurenteric canal is here considerably less than either in front or behind. A reference to the ventral surface-view (Fig. 5a) shows that the ventral opening of the neurenteric canal is dumb-bell shaped.

g is seven sections behind *f*. The ventral opening of the neurenteric canal is just beginning to close, while it is clearly open above. The wall of the neurenteric canal is

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now continuous by its whole height with the mesoblast. Its connection with the epiblast above, and with the hypoblast below, remains the same as before.

h is two sections behind *g*, and is the first section behind the closure of the ventral opening of the neurenteric canal. The canal is also just beginning to close above. This takes place a little below the surface of the epiblast, so that the latter layer is marked by a groove in the median line.

i is three sections behind *h*. It is the last section in which the posterior wall of the neurenteric canal is visible in section. The median superficial groove, *i.e.*, the primitive groove, is well marked, and on each side of it the epiblast dips down to become continuous with the mass below. It may be pointed out here that the height of the sections, *i.e.*, the distance between their dorsal and ventral surfaces in the median region, has been increasing from *c* backwards and is greatest in *h* and *i*. A reference to the dorsal and ventral surface-views shows that there is a considerable thick mass, the "Endwulst," around the neurenteric canal. The height decreases again from this point.

j is five sections behind *i*. The well-known characteristics of the vertebrate primitive streak are very distinct in this section. We must consider this as about the end of the "Endwulst."

k is four sections behind *j*, and shows all the points of a typical section through the vertebrate primitive streak. The height is considerably less than in *j*, *i*, or *h*. The groove is deeper.

l is four sections behind *k*. The primitive groove has flattened out. There is now only a trough-like depression over the epiblast, with the deepest point in the median line. Over this trough and without any relation to any adjacent structure there is an irregular granular

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mass with a few nuclei. This is the anterior free point of the yolk-plug. The epiblast is also changed in character for a short extent in the median line, being more granular, with nuclei scattered irregularly. It is very difficult to decide whether there is any connection between the epiblast and the mesoblast in the section, but if it is present, it is certainly very slight. On the whole, I consider that there is no such.

m is five sections behind *l* and is the fourth of five similar sections through the yolk-plug. The latter rises up from below between what can be recognised as the lips of the epiblast. Its similarity to Series I., *f*, or Series III., *k*, is so great that it is only necessary to call attention to it. The hypoblast is no longer a cellular layer in this section ; it consists of a protoplasmic network with yolk-spherules and nuclei imbedded in it.

n is four sections behind *m*. In tracing the yolk-plug backwards from such a section as *m*, we find it fusing laterally with the epiblast but its place can be identified, as it is coarsely granular and is also arched above, at first very distinctly, then gradually less and less. In this section, its place is indicated by two stars : it is already only slightly arched. The layers are independent. One or two sections behind, arching becomes hardly recognisable.

In this *Chelonia* embryo, the primitive streak stretches from the region of the neurenteric canal to the yolk-plug. The primitive groove also begins at the posterior part of the medullary canal which is still open dorsally, and reaching over the entire length of the primitive streak, widens out at the posterior end to allow the yolk-plug to stick out between them. The yolk-plug is very distinct and unmistakable, but its posterior limit can not be fixed definitely.

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Series VI.

(Pl. V.)

Trionyx japonicus.

An embryo with about 10 mesoblastic somites, taken out 8 1/2 days after deposition; represented in Fig. 22 (Pl. III.) and similar to Contrib. II., Pl. III., Figs. 19 & 19a. (143 田)

a is from the hinder portion of the mid-dorsal region. The medullary chord shows the first sign of fusion with the noto-chord by the presence of two nuclei lying crosswise in the boundary between the two chords.

b is two sections behind *a*. The two chords have now fused with each other.

c is thirteen sections behind *b*. The two chords, the hypoblast, and the mesoblast of the right side are fusing with one another.

d is two sections behind *c* and is the first in which the neurenteric canal is open below. The mesoblast of the left side is still independent,—due no doubt to the obliquity of the section.

e is six sections behind *d*. The wall of the neurenteric canal is now fused with the mesoblast by its whole sides.

f is next to *e*. The neurenteric canal is just closed. Otherwise it is like *e*.

g is three sections behind *f*. It is out of the region of the neurenteric canal. In the median dorsal line, the epiblast appears to be connected with the mass below for a short space. There is no primitive groove in the median line. This corresponds to Series III., *i*, or to Series V., *i*.

h is eight sections behind *g*. In passing from *g* to *h*, the "Endwulst" has been gradually diminishing, and in *h*, is

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no longer present. The primitive streak which is continuous from *g* backwards is now decided and pronounced. On the right (apparent left) above the streak and resting on the epiblast is the anterior termination of the yolk-plug. It gradually thins out like the blade of a razor towards the left (apparent right).

i is two sections behind *h* and is in the region of the yolk-plug. Resting on a trough-like depression of the epiblast in the median dorsal line is a triangular mass with a very broad base upwards. This is the yolk-plug. It is continuous directly with the mesoblast below and with the epiblast laterally. Although somewhat different in details, we must regard this section as corresponding to Series I., *f*, Series III., *k*, and Series V., *m*.

j is two sections behind *i*, and is nearly out of the region of the primitive streak and the yolk-plug. There is a slice of the latter on the left (apparent right) and there is still a slight connection between the epiblast and the mesoblast. The layers become independent after this.

This series shows that although there is no primitive groove in *Trionyx*, the primitive streak is really present, as in *Clemmys* and *Chelonia*.

Accompanying the above series of transverse sections is given a median longitudical section of the posterior portion of an embryo (*Series VI. long*, Pl. V.) taken out at the same time from the same deposit as above. In this, I was able to recognise the yolk-plug, which is apt to elude us in longitudinal sections. The "Endwulst" mass is shown to be tolerably well-marked at least posteriorly. The mesoblast on each side of the "Endwulst" in *e*, *f*, and *g* of the transverse series goes also *round the posterior end* of the "Endwulst" and is thus visible in the longitudinal section behind that structure. The lines through this section indicate by the lettering

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the planes corresponding to the transverse sections marked with the same letters in the above series.

Series VII.

(Pl. VI.)

Trionyx japonicus.

A Trionyx embryo, taken out 7 1/2 days after deposition, represented in Fig. 24 (Pl. II.), in general appearance very much like Figs. 22 or 23. (155 □).

This, it may be remembered, is one of those embryos in which a most diligent search failed to reveal a yolk-plug (see p. 29). Only one section of this series is introduced here.

a is from the region where the yolk-plug ought to be present. It will be seen to be essentially like Series VI., *i*; except that the groove separating the yolk-plug from the epiblast on each side is not so distinct as in that section. This is undoubtedly the reason why I was unable to recognise the yolk-plug in the surface-view. We may, therefore say, I think, without much straining, that the yolk-plug or what corresponds to it is in reality present in this embryo as in those in which the yolk-plug is plainly visible on the surface.

A study of the whole series shows that in that part which corresponds to Series VI, *g*, the epiblast has become already independent of the "Endwulst" mass below and that the primitive streak is found only in the hinder part beginning at the posterior limit of the "Endwulst." In this embryo, the rising of the tail swelling is also begun. Of this process, however more will be said in the sequel.

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Series VIII.

(Pl. VI.)

Trionyx japonicus.

A Trionyx embryo with about 13 mesoblastic somites; taken out 9½ days after deposition; represented in Figs. 25 (Pl. III.) & 25a (Pl. II.). (151 田)

This is one of the embryos in which the tail-swelling is beginning to arise, as explained on p. 30.

a is from the region slightly in front of the neurenteric canal. Not only is the medullary chord united with the notochord but the gut hypoblast also.

b is eight sections behind *a* and is the sixth in which the neurenteric canal is open below. It is already near the posterior end of the ventral opening. The mesoblast is united on either side for a short stretch with the wall of the neurenteric canal.

c is two sections behind *b*. The neurenteric canal has already closed in the section next in front. The figure of the medullary chord is, however, still recognisable in the "Endwulst" mass as a median dorsal projection. The epiblast is specially thinned over this projection but already there is no connection between the two in *Trionyx*. On each side of the median projection, there is a large mass of cells which is sharply distinguished from the lateral part of the middle layer by a shoulder-like slope. This shoulder-like mass, together with the median projection and the mass continuous with the gut-hypoblast below, form one compact central mass, which is sharply distinguished from the lateral parts. This is the "Endwulst," and corresponds to the innermost of the three concentric zones in the superficial view of the embryo (Fig. 25). It is this mass already risen up some-

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what and destined to rise still more that is to form the tail-swelling. On comparing this section with *b*, it is found that the shoulder-like mass in *c* corresponds to that part of the mesoblast in *b* which is marked off as the mesoblastic somites. The lateral plates of the mesoblast are therefore out of the area of elevation.

d is nine sections behind *c*. The "Endwulst" mass is smaller than in *c*, but stands out more prominently and shows more distinctly that it is rising up. The lateral plates of the mesoblast are not included in the elevation and are left below. The foregoing section and this one show that the *tail-swelling is not produced by folding, but by the growth upwards of the "Endwulst" mass.* If it were produced by folding, the lateral plates, for one thing, ought to be near the dorsal surface of the "Endwulst" instead of being down below, as in this section. But of this more in some of the following series. The epiblast is very much thickened outside of the "Endwulst." The middle of the three concentric zones of the superficial view, (Fig. 25), I believe, corresponds with this thickened zone of the epiblast.

e is three sections behind *d*. The great thickness of the epiblast in this section is due to the fact that the thick wall of the epiblast such as is shown on each side of the tail-swelling in *d* goes entirely around its posterior end and this section just goes through this wall. Note that in all the sections in front of, and including, this, the epiblast is entirely independent of the layers below. A comparison with Series VI shows that the primitive streak, which extended in an earlier stage *over* the "Endwulst" (VI, *g*.), has now disappeared from that part. Of this, more anon.

f is two sections behind *e* and is the second in which the epiblast is united with the layers below it.

g is two sections behind *f*. The coelem now runs nearly across, dividing the mesoblast into the parietal and vis-

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ceral layers. *f* and *i* are through the region of the yolk-plug and that part included between the stars must be considered as a rudimentary form of the yolk-plug, like that in Series VI, *i* or Series VII, *a*. In *g*, there is a very slight groove on each side.

On tracing the series still further backwards, the triangular downward projection of the epiblast on each side becomes less and less, until the epiblast is of an uniform thickness across. The connection of the epiblast with the mesoblast is given up some sections before that.

Series IX.

(Pl. VII.)

Chelonia caouana.

A Chelonia embryo with about 16 mesoblastic somites; taken out 10½ days after deposition; represented in Figs. 8 (Pl. I.) & 8a (Pl. II.)

(B12 1)

This is one of the most important series. The tail-swelling is fully arisen, and the yolk-plug is very distinctly visible behind it, as a white mass.

a is from the posterior dorsal region and shows the first sign of the fusion of the notochord and the medullary chord by the presence of some spindle-shaped cells lying transversely at the boundary between the two chords.

b is eight sections behind *a*. The notochord and the medullary chord are fused together.

c is next behind *b*. The notochord shows division into two halves, and also is continuous with the gut-hypoblast.

d is next behind *c* and is the first section in which the neurenteric canal is open below. Note that the *chorda-*

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"Anlage," although divided into two shows a distinct *hemi-chorda* on each side.

e is nine sections behind *d*. The neurenteric canal is just closing. The mesoblast is not distinctly separated from the wall of the neurenteric canal but is specially continuous with the *chorda*-part of the neurenteric wall.

f is next behind *e*. The neurenteric canal is now clearly closed below. The mesoblast is fused with the wall of the canal along its whole height.

g is four sections behind *f*. The dorsal median part of the section which has been more and more vaulting from *a* backwards is now clearly recognisable as the beginning of the tail-swelling. The continuation of the neurenteric canal *is open above* (this opening begins in the section next in front) *and the epiblast is reflected downwards at the edge of the opening to become continuous with the mass below*. The wall of the neurenteric canal, although clearly recognisable as such by the arrangement of their cells, is amalgamated with the rest of the "Endwulst" mass.

h is four sections behind *g*. It is behind the posterior wall of the neurenteric canal and shows the last vestiges of its wall. There is a median dorsal groove—the primitive groove—continued backwards from the dorsal opening of the neurenteric canal (*g*). At its lips, the epiblast is reflected downwards and becomes continuous with the mass below.

i is three sections behind *h*. The tail-swelling is more and more distinctly recognisable as such, rising as a nearly separate body above the general surface of the blastoderm. The mass within—the "Endwulst"—is compact and homogeneous; it is united dorsally at the lips of the primitive groove with the epiblast on each side, and ventrally by a rather broad stalk with the mass below. Note that the lateral plates of the mesoblast are below and do not take part in the formation of the tail-swelling.

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j is two sections behind *i*. We are now near the posterior end of the tail-swelling. The primitive groove is still present and at its edges the epiblast is reflected downwards to become continuous with the mass within. The latter is connected by a small string of cells with the mesoblast below in which there is now visible a line running across from side to side through its middle, dividing it into the parietal and visceral layers.

k is next to *j*. The posterior end of the tail-swelling is now reached. As that part is cut tangentially, it is very hard to distinguish the epiblast from the enclosed "Endwulst" mass. The upper part is undoubtedly the epiblast; and the lower part, crowded with cells, has probably the two elements represented, although it is impossible to fix the limits. The primitive groove is distinct. Below the tail-swelling, the epiblast now goes entirely across from side to side. The tail-swelling, the epiblast, and the mesoblast seem to be united together in the median line.

l is two sections behind *k*. The very tip of the tail-swelling is cut tangentially. The fact that the tail appears as *two* masses is due to the circumstance that the primitive groove goes from the dorsal surface around the tip of the tail to the ventral surface, and this section has cut the groove longitudinally at the tip. The two masses consist evidently of the epiblast. The epiblast below the tail is very granular in the median line. I can not make myself sure whether this granular part is continuous with the mesoblast below or not. On the whole it seems to be not continuous.

m is next to *l*. We are now clear of the tail-swelling, and in the region of the posterior tube of the amnion. Hence, there is only a clear tube without any structure enclosed in it. I can not be certain whether there is any connection between the epiblast and the mesoblast in the

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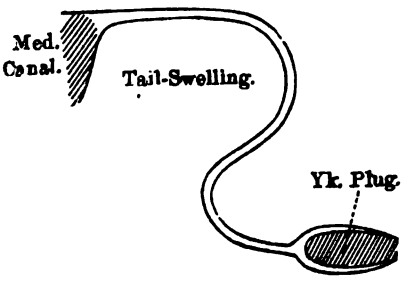
median line of the floor of the posterior amniotic tube. They are suspiciously close at two points, so much so at one of them (marked by an arrow) that the layers can not be clearly distinguished.

n is the third section behind *m*. The yolk-plug is distinct and unmistakable and projects out above the epiblast on the floor of the amniotic tube. Here is a connection between the yolk-plug and the mesoblast in the median line. The yolk-plug begins in the section next behind *m*, and is found in five sections including that one. *n* is therefore the third or the middle one of the five. For some sections after *n*, the median line of the epiblast is arched upwards, marking the posterior prolongation of the yolk-plug on the epiblast. Moreover, there seems to be a fusion, in the median line, of the epiblast with the two layers of the mesoblast, and thus the coelem does not go across from side to side, being interrupted by the median partition.

Although the primitive streak can not be clearly made out in the region of *l* and *m*, I think, we may consider this as due to the fact that the whole structure is more or less rudimentary. The primitive streak and the primitive groove in this specimen may therefore be considered to begin at the last part of the medullary canal (*g*), then to run backwards along the dorsal surface of the tail-swelling (*g-k*), around and down its posterior tip to the ventral surface (*k-l*), and then backwards along the median line of the epiblast below the tail-swelling (*k-m*), until it reaches the yolk-plug at its posterior end (*n*). Diagram-

Woodcut VII.



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matically represented, the figure of the primitive streak and groove will be as in Woodcut VII. This is exactly like the figure of the blastopore which SEDGWICK figures in his notes on the Elasmobranch ('92, p. 564). The significance of this will be discussed later on.

Series X.

(Pl. VIII.)

Clemmys japonica.

An embryo with about 10 mesoblastic somites; taken out 6½ days after deposition. (LI. ⊞).

This embryo was taken out at the same time from the same deposit as that represented in Fig. 14, but it is greatly different from that figure in regard to the backward progress of the amnion. That membrane has not only extended over the entire embryo but produced itself backwards for a distance equal to about one-third of the length of the embryo as the posterior tube of the amnion, which remains very wide in this specimen, being hardly reduced in width compared with the part over the posterior part of the embryo. The ventral view of the embryo is very much like Fig. 5*a*: only the shape of the ventral opening of the neurenteric canal is spindle-shaped, instead of being dumb-bell shaped, as in that figure.

This is considerably younger than the *Chelonia* embryo of Series IX. It and the next series are introduced mainly to illustrate the process of the upheaval of the tail-swelling.

a is from the posterior part of the dorsal region. The medullary chord and the notochord show the first signs

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of fusion, as evinced by spindle-shaped cells lying at the boundary of the two.

b is eight sections behind *a*. The medullary chord, the notochord, and the hypoblast are united. The notochord shows a division into two halves.

c is two sections behind *b* and is the first in which the neurenteric canal opens below. The two halves of the notochord, although separated, are recognisable as such. The mesoblast has united on each side with the *chorda dorsalis*.

d is seven sections behind *c* and is the last in which the neurenteric canal is open below. The mesoblast has now united with the wall of the neurenteric canal along its whole height.

e is two sections behind *d*. We are now in the "Endwulst" region. The posterior part of the neurenteric canal is still present.

f is two sections behind *e*. The last vestiges of the neurenteric canal is visible in the upper median part of the "Endwulst."

g is three sections behind *f*. The "Eudwulst" is simply a compact mass of indifferent cells. It is united with the lateral mesoblast *by its whole height*, and with the hypoblast below; it is independent of the epiblast.

h is five sections behind *g*. The "Endwulst" mass is smaller. It also projects upwards above the level of the lateral mesoblast. We must regard this as the first sign of the upheaval.

i is three sections behind *h*. The median "Endwulst" mass is still less than in *h*.

j is next to *i*. The epiblast which has hitherto been independent, is now continuous with the last part of the "Endwulst" and the mesoblast. That is, the primitive streak which began in Series III from the neurenteric canal, begins here in this series.

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k is six sections behind *j*. There is a distinct median groove, at the lips of which the epiblast bends down to become continuous with the mesoblast. The groove begins in the second section after *j* and is already deep in the fourth section.

l is five sections behind *k*. The groove is shallower and not so marked as in *k*.

m is seven sections behind *l*. The yolk-plug appears in the groove, first in the fourth section behind *l*. In this section, it is very well marked and unmistakable.

n is five sections behind *m*. The yolk-plug has gradually united with the epiblast on both sides, but its place is still marked by a slight arching upwards (indicated by two stars in the figure) and by the fact of its being united with the mesoblast below. After three or four sections, the connection of the epiblast with the mesoblast is lost, but *the median elevation of the epiblast marking the posterior prolongation of the yolk-plug is to be seen in this series for thirty sections or more.*

Accompanying this series of transverse sections is given the median longitudinal section of another embryo taken out from the same deposit at the same time (*Series X, long*, Pl. VIII.). It shows nothing not seen in the transverse series, and only serves as a check on it. The lines through this section show by their letters the levels of the transverse sections indicated by the same letters.

In this *Clemmys* embryo, the sections show that the tail-swelling is not yet much raised. The primitive streak, which in earlier stages began from the posterior end of the medullary canal (Series III), has now disappeared from over the "Endwulst," but behind this mass, there are the streak and the groove, with the yolk-plug at the posterior end. The posterior prolongation of the yolk-plug is very long, but of this more anon.

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Series XI.

(Pl. IX.)

Clemmys japonica.

An embryo with about 13 mesoblastic somites; taken out 8 days after deposition; like that represented in Fig. 15 (Pl. II.). (XXXXIII ⊕)

The tail-swelling is very distinctly arisen. The posterior tube of the amnion is produced to a length about two-thirds that of the embryo proper.

a is from the posterior dorsal region. The medullary chord and the notochord show the first sign of fusion by the presence of a few spindle-shaped cells at the boundary between the two.

b is ten sections behind *a*. The medullary chord, the notochord, and the hypoblast are united together.

c is two sections behind *b*, and is the first in which the neurenteric canal is open below. Two *hemi-chordae* are distinctly recognisable.

d is five sections behind *c*. The posterior limit of the neurenteric canal is reached and it is just closing. The mesoblast of both sides is united with the wall of the neurenteric canal. Note that, from *a* backwards, the dorsum of the sections is much more arched than in Series X. This is necessary in order to pass to the now elevated tail-swelling.

e is four sections behind *d*. The posterior part of the neurenteric canal is still distinct. The middle portion of the section enclosing the "Endwulst" is quite high.

f is two sections behind *e*. The last vestiges of the wall of the neurenteric canal are present.

g is next behind *f*. The "Endwulst" is now homogeneous, except for a crowding of cells in its middle caused by the very last vestiges of the wall of the neurenteric canal.

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h is four sections behind *g*. The tail-swelling is now circumscribed by the epiblast all around, except on the ventral surface. The "Endwulst" mass within is now quite homogeneous and is continuous with the layers below only by a stalk which is, however, still rather broad. *Note that, in this as well as in the preceding and following sections, the lateral plates of the mesoblast do not take part in the tail-swelling and are left below.*

i is two sections behind *h*. The stalk of the "Endwulst" is now rather narrow.

j is three sections behind *i* and is nearing the posterior termination of the tail-swelling. It is becoming difficult to distinguish the mass within from the epiblast—not because there is not really a limit, but because the section cuts these parts somewhat obliquely near the rounded termination of the tail. A comparison with Series IX shows that, unlike *Chelonia*, the primitive streak and groove are early obliterated from the dorsal surface and the tip of the tail-swelling in *Clemmys*. The primitive streak below the tail-swelling seems to begin at about the level of this section.

k is two sections behind *j*. It has just scraped the tip of the tail-swelling. There is a distinct primitive streak in the epiblast below. The nuclei are arranged in two rows in the stalk that connects the epiblast and mesoblast, and there is a distinct cavity between the two rows. I think, we may regard this as a very deep primitive groove which is closed above near the surface by the coalescence of the lips.

l is two sections behind *k*. We are now behind the tail-swelling, and in the posterior tube of the amnion. The primitive streak is distinct. Although the median part of the floor of the amniotic tube is elevated, I do not think it can be regarded as the yolk-plug which, I believe, is found further behind.

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m is three sections behind *l*, and is very much like that section : only the floor of the posterior aminotic tube is rather irregularly warped and the coelom has extended across from side to side, dividing the mesoblast into the parietal and the visceral layers.

n is three sections behind *m*.

o is six sections behind *n*. From the third section in front of *o* (inclusive), a median knob begins to show itself on the floor of the posterior amniotic tube and assumes in one or two sections the shape represented in this one. There is a slight connection between it and the mesoblast. I believe we ought to regard *this* as the yolk-plug. The remarkable fact is that this elevation continues *for seventy sections* or *more;* in fact it extends a little beyond the termination of the posterior amniotic tube and is visible in the surface-view as a white streak between the limbs of the horse-shoe shaped opening of that tube (Fig. 15). The elevation is, in the middle portion of the tube, even higher than in *o*, occupying almost the whole of the somewhat small lumen of the tube, but becomes broad and flat, as the tube flattens near the posterior end. The connection with the mesoblast seems to be given up rather early, although I can not make out exactly where it takes place.

A comparative study of this and Series X throws light on the process of the formation of the tail-swelling. It is often stated in works on Embryology that the tail is formed by the tucking in of the blastoderm under what is going to be the tail, *i.e.*, by a process of folding. Such is, however, not the case in Chelonia. In Series X, the tail-swelling is hardly marked, while it is fully formed in this series (XI). A comparison of this series *e–j* with Series X, *e–i* shows that a large mass which is not present in the latter series has been added to the dorsal surface of the sections in the former series,

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and that the parts which are found in the sections of Series X are all confined *to the lower part of the sections in Series XI.* This shows that the tail is formed by a new *elevation* or *upheaval* and not by the tucking in of the blastoderm under something which previously existed. If the latter process had taken place, the parts found in the sections of Series X ought to be *near the dorsal surface, and not left far below, in Series XI.* A diagram will make this point clear. Let *A B* in Woodcut VIII represent the original level surface of the blastoderm. Then the tail is not formed by tucking in as shown by the line *CDE*, but by the gradual elevation given by the lines *FC*, *GC*, and *HC*.

Woodcut VIII.

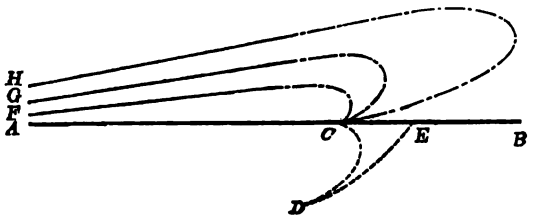

Diagram to illustrate the formation of the tail.

I wish to add a few words in regard to the median ridge on the floor of the posterior amniotic tube. Of the three species I have studied, *Clemmys* alone presents this feature. In *Chelonia* and *Trionyx*, so far at least as I have examined them, the yolk-plug stops always within reasonable distance. But it seems to be a characteristic of *Clemmys* that what appears to be the posterior prolongation of the yolk-plug attains such a length as that described in this Series (*o*). I am unable to see its exact significance, but it seems reasonable to suppose that it has arisen with some relation to the yolk-plug. MEHNERT ('95) has seen it in *Emys lutaria taurica* (Taf. X. Fig. 26) and gives an entirely different explanation of the structure. From what has been stated above it will not be surprising that I find myself unable to accept his views.*

* In passing, I may remark that I have never found in the species I have examined any structure which I can identify with, or can consider as resembling, MEHNERT's "retroembryonal Ectoderm diverticulum."

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Series XII.

(Pl. VI.)

Trionyx japonicus.

An embryo with about 15 mesoblastic somites; taken out 10½ days after deposition; represented in Figs. 26 (Pl. III.) and 26a (Pl. II.).

In this embryo, not only is the tail-swelling already well developed but the allantois has made considerable progress in development.

a is in front of the anterior end of the neurenteric canal. The medullary chord, the notochord, and the hypoblast are already united together.

b is three sections behind *a* and is the second section in which the neurenteric canal is open below. The mesoblast is united with the wall of the neurenteric canal for a short extent

c is five sections behind *b* and is the first in which the neurenteric canal is closed below. The wall of the canal is still recognisable, although united with the mesoblast along its whole height.

d is five sections behind *c*. There is still a trace of the wall of the neurenteric canal in the most dorsal part of the "Endwulst." There is a bay in the hypoblast.

e is two sections behind *d*. The "Endwulst" mass is divided into two masses, an upper and lower. The upper is the tail itself and the lower the allantoic mass. In the latter, there is a deep bay from the enteron below.

f is two sections behind *e* and is very much like that section, but the allantoic bay is now cut off from the enteron below. Note that in *c–f*, the lateral plates are below and not included in the tail-swelling.

g is two sections behind *f*. The upper mass of the "End-

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wulst" is now circumscribed entirely by the epiblast and forms the tail. The lower allantoic mass lies almost free in the lower part. The coelem goes across from side to side.

h is two sections behind *g*. The tip of the tail is just scraped. The lower mass, which is the continuation of the allantoic wall, is now changed to the stalk that connects the epiblast below the tail with the mesoblast. This is comparable to Series XI, *j* or *k*. (Pl. IX.).

i is two sections behind *h* and takes in the groove surrounding the tail; hence the primitive streak is cut more or less obliquely and longitudinally. (*Cf* the surface view Fig. 26).

j is next to *i*. We recognise in the median line the mushroom-like yolk-plug visible in the surface view. Unlike *Clemmys* (Series X and XI), the yolk-plug is recognisable only in three or four sections.

Accompanying this is the longitudinal section (*Series XII, long.*, Pl. VIII.) which will make the relations of different parts clearer.

Series XIII.

(Pl X.)

Chelonia caouana.

An embryo with about 18-19 mesoblastic somites; taken out 11½ days after deposition; in general appearance very much like the Clemmys embryo given in Fig. 15 (Pl. II.). (B13¤)

In this embryo, the tail has much elongated and the allantois has grown somewhat. This is the most advanced stage in which I have been able to recognise the remnant of the yolk-plug distinctly.

a is from the posterior dorsal region.

b is four sections behind *a*. The medullary chord and the

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notochord show signs of fusion by the presence of some spindle-shaped cells in the boundary between the two.

c is two sections behind *b*. The enteron, though still open, is narrowing. The medullary chord and the notochord are now nearly fused.

d is next to *c*. The enteron is closed below, and shows a partial division into the upper enteron proper and the lower allantoic cavity.

e is next to *d*. The division is now complete.

f is next to *e*. The cavity has disappeared from the allantois.

g is next to *f*. The noto-medullary-chord shows signs of fusion with the wall of the enteron. The mesoblast on each side of the enteron is fused with the posterior continuation of the allantoic mass. The coelom has gone across.

h is two sections behind *g* and is very much like that section. Only the epiblast has nearly encircled the tail which is unenclosed only in the ventral median line.

i is two sections behind *h*. The tail is now entirely encircled by the epiblast and lies free in the amniotic tube. At the point where the epiblast of two sides met last in the median ventral line of the tail, the epiblast is fused with the mass within. This is evidently a part of the primitive streak. The cavities of the enteron and the medullary chord are united as the neurenteric canal. The lower part of the enteric wall is fused with the indifferent cell-mass. In the amniotic tube surrounding the tail, the epiblast is clear all around of the mesoblast lining it, and thus there is no primitive streak here.

j is three sections behind *i*. The enteron has become lost in the indifferent cell-mass and only the medullary chord is still persistent, although the lower part of its wall is already fused with the indifferent blastema. The primitive streak is still present in the ventral median line of the tail.

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k is seven sections behind *j*. The mass within the tail shows some slight trace of the medullary chord in its upper dorsal part. The primitive streak is still persistent, although it is not as distinct as in front. This is the last section in which the primitive streak is visible.

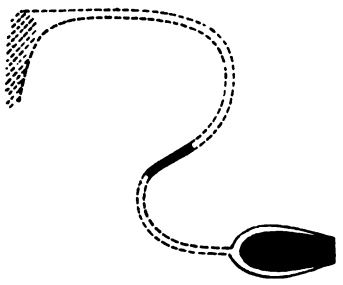
l is next to *k*. The tail now consists only of the indifferent cell-mass surrounded by the epiblast on the outside.

There are five sections behind *l* in which the tail is visible, although gradually reduced in circumference.

In the fourth section behind *l* there is a structure standing up from the floor of the posterior amniotic tube which is figured in Series XIII *m* (Pl. X.). This is so similar in appearance and position to the yolk-plug observed in earlier stages that we must conclude that here we observe its remnants. This is found in six sections, in three of which it is quite prominent. The one figured is the second of the three prominent sections and the second of the six. This is the last stage in which I was able to detect the yolk-plug in sections.

Of the primitive streak which was present in earlier stage (Series IX and Woodcut VII), only a small portion in the ventral median line in the anterior part of the tail remains at this stage. The formation of the tail by upheaval easily explains the presence of a part of the primitive streak in the ventral median line. If Woodcut VII (p. 56) represents the extent of the primitive streak at the stage of Series IX, we may consider Woodcut IX as representing the state of things in the present series. The dotted part has disappeared and only the part given in the full line, together with the yolk-plug, now remains. The proctodæum seems to be formed

Woodcut IX.



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in the part of the primitive streak which persists, as will be seen in the next series.

In the elongation of the tail, various structures are developed *in situ* from the indifferent "Endwulst" cell-mass. Of these, the medullary chord is formed first (*j*), and then the enteron and the notochord are differentiated (*j-i*), although the lowest part of the enteron is fused with the indifferent tissue for some time after the rest has been formed. The mesoblast is also formed from the indifferent tissue.

Series XIV.

(Pl. XI.)

Chelonia caonana.

A quite advanced embryo taken out 16 days after deposition; with five gill-slits all formed, with the cranial flexure well marked, with the eyes and ears already quite advanced, and with the appendages present as knobs. About the stage of Figs. 11 and 11a, Contrib. II. The allantois appears in the body cavity as a small vesicle. (F7a)

This series is introduced to show the relations of different parts in the posterior part of an advanced embryo, and to show the formation of the proctodæum.

a is from the posterior dorsal region. The allantois is separate from the embryo proper.

b is three sections behind *a*. The allantoic mass has united with the body proper on the left side.

c is four sections behind *b*, which it very much resembles.

d is seven sections behind *c*. The allantoic mass is now united with the body proper on both sides. The allantoic cavity is gradually approaching the enteron.

e is three sections behind *d*. The epiblast is gradually enclosing the body proper.

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f is four sections behind *e*. The enclosure has gone one step further.

g is two sections behind *f*. The enclosure is now complete, but the epiblast is still continuous with the epiblast of the extra-embryonic blastoderm. The epiblast is thick at this point of reflection.

h is two sections behind *g*. The enteron has now united with the allantoic cavity.

i is two sections behind *h*. The connection of the epiblast at the ventral median line is now cut and the tail lies entirely free in the amniotic cavity.

j is seven sections behind *i*. It is in the region of the cloaca. The Wolffian ducts empty into the cloaca in this and next section.

k is next to *j*. The ventral median part of the tail is given on a larger scale in Ser. XIV *k'*. on the same plate. The three layers are distinctly separate.

l is eight sections behind *k*. The ventral median part of the tail is again given on a larger scale in Ser. XIV *l'* on the same plate. Here the wall of the enteron is distinctly united with the epiblast, and there can not be much doubt that this is the *proctodæum*. Note how the two cell-layers of the epiblast behave in this section. The section in front of, and the two sections behind, *l* are like *l*, and the proctodæum extends thus over four sections, but for some five sections further forwards the epiblast shows some thickening in the ventral median line which is the greater, the nearer to the proctodæum. Behind, the connection of the epiblast with the enteric wall is loosened at the third section behind *l*.

m is six sections behind *l*. The enteron is contracted very much immediately after the level of the proctodæum. In this, only the upper part has a lumen.

n is two sections behind *m*. The enteron is now reduced to

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a few cells. The two sections following *n* show similar groups of enteric cells.

o is the fourth section behind *n*. There is no trace whatever of the enteron in this. For thirteen sections, this condition continues; then just for two sections, viz., on the fourteenth and fifteenth sections behind *o*, of which—

p represents the latter, there is a group of a few cells which from their position and manner of deeper staining can be nothing else than hypoblast cells. The cell-string again disappears for seven sections. Then, on—

q the eighth section behind *p*, the cell-group again appears and this time is continued to the end of the tail, gradually enlarging in size as the following sections show.*

r is twenty-three sections behind *q*. The hypoblast string is slightly larger than in *q*. The upper part of the mesoblast shows an arrangement like the mesoblastic somites but is merged below with the indifferent mass, without any limitation.

s is eleven sections behind *r*. The hypoblast string is larger.

t is fourteen sections behind *s*. The hypoblast mass is now quite large and there is a lumen in its upper part. The mesoblast has largely acquired the epithelial arrangement, but there is still a part below where stellate cells are scattered.

u is nine sections behind *t*. In this section, there is no longer any stellate cells below. The enteron is not painted black from this section on.

v is two sections behind *u*. The notochord and enteron have united, as also the enteron and mesoblast below.

w is the third section behind *v*. The medullary canal, the notochord, and the enteron are now united and a narrow slit running through the middle of the first two empties into the relatively large enteric lumen. This can be no other than the neurenteric canal. The enteron is united below with tne mesoblast.

* The scale of enlargement differs from this section onward.

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x is two sections behind *w*. All the parts enclosed within the epiblast of the tail are now united, but a peculiar lobe-like projection on the dorsal side indicates where the medullary canal ought to be, and the two slits mark the boundary between the mesoblast and hypoblast.

y is two sections behind *x*. The mass within the tail would be circular in outline but for a projection on the dorsal side.

z is two sections behind *y*. The mass within now consists entirely of an indifferent tissue, without any sign of differentiation into parts.

This series shows that the proctodæum is formed within the limits of the primitive streak, namely, in that part which runs from the tip of the tail forward to the stalk of the embryo.

The enteron behind the proctodæum rapidly narrows and is soon reduced to a string of cells (*n*); nevertheless it is continued to the tail end, *with two interruptions, however*, in this embryo (at *o*, and behind *p*). It would be reasonable to suppose that at an earlier stage, the hypoblast string suffered no interruption.

If we go gradually from *z* forwards, we see in what order the different parts are differentiated from the indifferent cell-mass within the tail, as the latter elongates behind.

C. SUMMARY OF THE FOREGOING FACTS.

From the account given above, it is evident that the mass which I have called the yolk-plug undergoes a series of very complex changes. Neither the view of WILL *, that it disappears from the surface when the blastopore lips close around it, nor that of MEHNERT, that it finally fuses laterally with the hindmost part of the medul-

* Which I accepted in Contrib. IV.

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lary folds and forms in the development of the tail its extreme tip, is tenable. It might be said perhaps that the yolk-plug may behave differently in different species. But I have worked its history out in three species, and it seems incredible to me that in any species it will undergo modifications that seem to have no genetic relation with those I have sketched out above.

It may not be out of place to summarise here the facts given in the preceding pages, and to give a partial discussion of them. The consideration of their theoretical significance I shall reserve for the next section.

At the very earliest stage of development, there is in the posterior part of the embryonic shield an area in which the primary layers are not differentiated—the primitive plate or knob. When the process of gastrulation begins, it takes place within this area. When this has occurred, all that part of the primitive knob *in front of* the blastopore is found to have differentiated itself into the epiblast and the primary hypoblast—these two layers being reflected into each other at the anterior lip of the blastopore. *Behind* the blastopore, however, there is still an area in which the layers are fused and over which the epiblast has not therefore spread itself. By the gradual change of shape in the blastopore (see Woodcut V), this posterior part is gradually hemmed in, and becomes surrounded, by the anterior lip of the blastopore. Thus, at a certain stage (Figs. 1 & 1*a*), the blastopore has assumed the horse-shoe shape and the posterior fused mass, free from the epiblast, is enclosed within the horse-shoe. This mass stands up, as it has always done, from the floor of the invaginated archenteron (or the blastoporic passage, when the archenteron has broken through below; see *Contrib. IV*). This is the mass which we have called the yolk-plug. Its relations to the germinal layers

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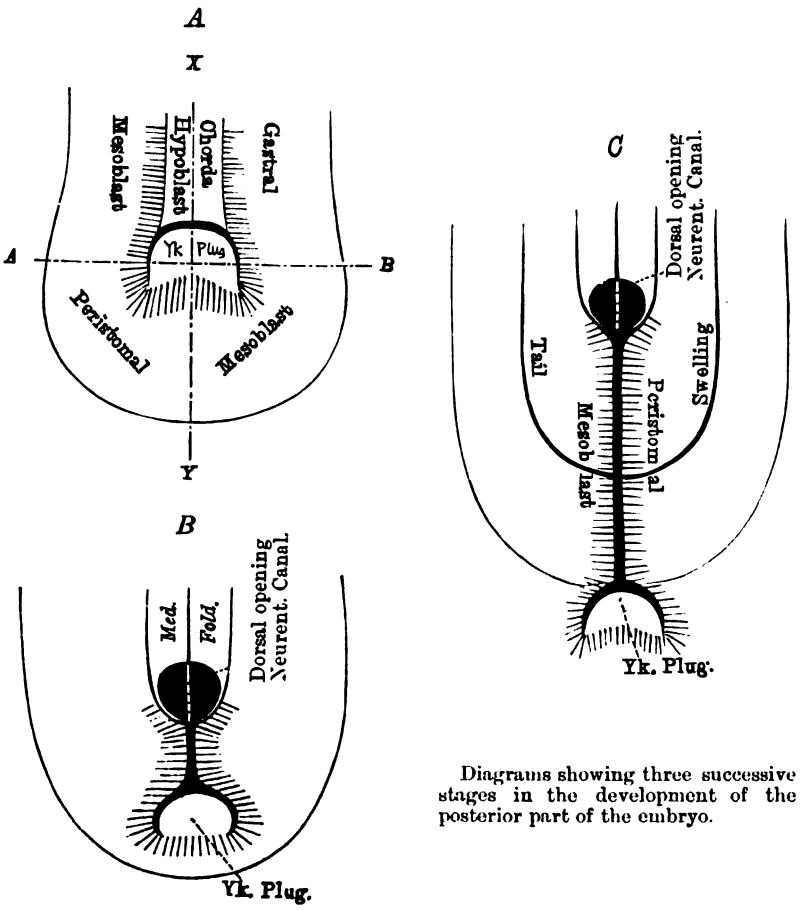
may be gathered from the accompanying diagrams. Woodcut X, *A*

Woodcut X.



Diagrams showing three successive stages in the development of the posterior part of the embryo.

gives the surface diagram and the line of origin of the gastral and peristomal mesoblast. The transverse section through the line *A B* in that diagram is given in Series I, *f*, and diagrammatically in Woodcut XI, *D*. The groove between the yolk-plug and the outer lip of the blastopore should ideally go into the midst of the mesoblast, dividing it into the parietal and visceral layers, and in some sections it does actually go much further into the mesoblast than in Series I, *f*. The

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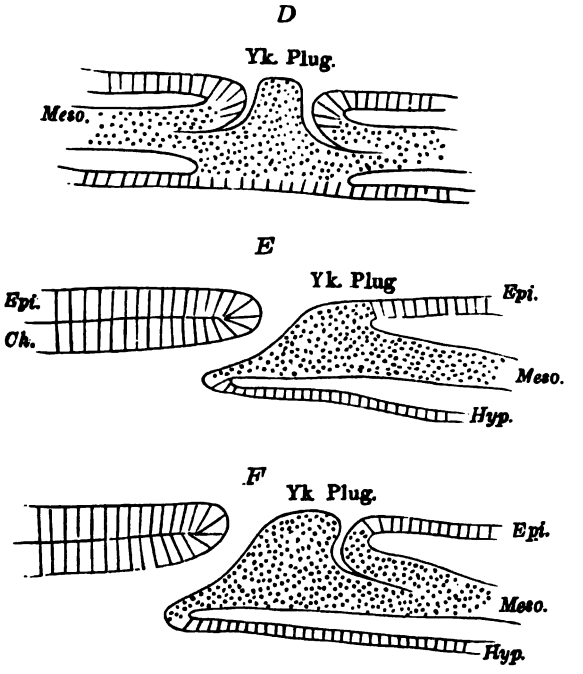
longitudinal section through the line *X–Y* (Woodcut X, *A*) is given in Woodcut XI, *E*. Actual sections have been figured several times in *Contributions I, III, and IV*. I have never seen a groove behind the yolk-plug, between it and the epiblast, but I believe that this is due to the rudimentary condition of the parts just here and that, theoretically, the section should be as in Woodcut XI, *F*. Thus, the peristomal

Woodcut XI.



mesoblast is proliferated partly from the cell-mass in question and partly from the epiblast, in the shape of an open fan from all around the yolk-plug except the anterior edge. At the edge of the anterior transverse part of the blastopore, the peristomal mesoblast is continued into the gastral mesoblast, for the details of whose origin the reader is referred to *Contributions I, and III.*

When the medullary folds arise and reach the posterior part of

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the embryonic shield, they embrace the yolk-plug between their posterior ends as in a vice, and compress it laterally, thus lifting it up to the level of their dorsal surface (Figs. 2, 9, 17, and 18). The yolk-plug meanwhile keeps its own independence.

The yolk-plug now apparently *recedes*, leaving in its track a groove from which the cells are proliferated. I have hitherto given only the objective description of this process, and have not discussed the nature of this apparent receding. I may now state that I believe this is caused by the active proliferation of cells from the *lateral blastopore lips, while pressing to meet each other in the median line.* As cells proliferated from the lips accummulate, they form a mass which necessarily pushes the yolk-plug backwards. This pushing goes on until the yolk-plug is placed at about the edge of the embryonic area. The edges of the groove (or track, as I have called it, left in the wake of the yolk-plug) must therefore be considered the lateral blastopore lips. Woodcut X, *A*, *B*, *C* will make this clear. *A* is the stage in which the yolk-plug has not yet begun its backward movement ; in *B*, it has moved some distance and, in *C* it has reached its final position. In the last stage, the blastopore therefore consists of three parts :—

(1) the dorsal opening of the neurenteric canal.

(2) the groove between (1) and (3).

(3) the groove around the yolk-plug in its final position.

With regard to the first of these three parts, *the dorsal opening* of the neurenteric canal (transformed from the blastoporic passage, which, in its turn, is directly derived from the posterior part of the archenteron) must of necessity be considered as a part of the blastopore, but it would be difficult in actuality to point out exactly where the *dorsal opening* lies, as it is merged beyond recognition in the posterior part of the medullary canal, though that it is there, is a matter of course. By most

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writers, the neurenteric canal *itself* is included with the blastopore, but this is a part of the original archenteron, and we must consider its *dorsal opening* alone as a part of the blastopore. It is highly significant that in such an advanced stage as that represented by Series IX or Fig. 8, the hindmost part of the neurenteric canal is open dorsally (*sect. g*) and becomes continuous with the groove which forms the second part of the blastopore. Of this second part, there is nothing special to say, since it so plainly is a part of the blastopore. In the third part, the groove is found only around the anterior part of the circumference of the yolk-plug, and I have never seen any visible posterior boundary. This absence I believe, is due to the yolk-plug being a rudimentary structure.

The line of cell-proliferation from the blastopore lips I have called the *primitive streak*, and the groove lying on it in the median line, the *primitive groove.*

In *Chelonia* and *Clemmys*, the blastopore lips do not become fused ; hence the primitive groove is distinct. In *Trionyx*, the groove is not present, but proliferation takes place all the same along the line where the groove ought to be

The anterior half of the primitive streak is much thicker than the posterior half and gives rise to the mass known as the "*End-wulst.*" The posterior part remains thin to the end. The former corresponds to the "vorderer dicker Theil (*ps. I.*)," and the latter to the "hinterer, dünner Theil (*ps. II.*)," of GASSER ('78).* The anterior thick part rises as the *tail-swelling.* This is formed by the addition of the new cell-mass on the dorsal surface, in other words by *upheaval or elevation and not by folding* (Series X and XI). The lateral plates of the mesoblast do not take part in this upheaval.

* It is a great pleasure to find my results agreeing in many points with the beautiful and accurate description by this author.

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As the tail is formed by the rising of the anterior thicker half of the primitive streak, the primitive streak and groove naturally cross the posterior boundary of the tail at right angles (Woodcut X, *C*). They should also of necessity be found over the dorsal median line of the tail, and, when the latter attains some height, around its tip on to the ventral median line, and then be continued to the thinner part of the primitive streak outside the embryo proper until they reach the yolk-plug. They are thus **2**-shaped. Such a condition is actually seen in *Chelonia* up to quite a late stage (Series IX). In *Clemmys*, the anterior part of the primitive streak and groove over the tail-swelling disappears rather early (Figs. 12, 13 & Series X, XI). In *Trionyx*, the primitive groove does not exist at all from the first, and the streak also disappears quite early from the tail part (Series VIII).

In the tail which lengthens itself backwards, the formation of the different parts of the embryo proceeds backwards, *i. e.*, the medullary chord, the notochord, the enteron, and the mesoblast are differentiated *in situ* in the tissue surrounded by the epiblast. As this is derived from the "Endwulst," we can not regard the latter solely as the mesoblast but as something *sui generis*.

The allantois is formed on the ventral surface of the "Endwulst" mass: hence its stalk is continuous with that mass.

The primitive streak finally disappears entirely by the separation of the layers, with the exception of a short stretch on the ventral surface of the tail. The proctodæum is formed at this point.

The yolk-plug, at the latest stage observed, stands out as an appendage of the epiblast (Series XIII). Probably it persists to a late stage as a rudimentary useless structure. In *Clemmys*, it is elongated posteriorly and forms on the floor of the posterior aminotic tube, a prominent ridge, the significance of which is not clear (Fig. 15 and Series XI).

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In the three species studied the blastoporic passage once formed is not closed and opened again, as affirmed by MEHNERT ('91) and WILL ('92 & '93) for *Emys*, *Platydachylus*, and *Cistudo*. It becomes directly transformed into the neurenteric canal.

Of the surface views given, Figs. 1–8 belong to *Chelonia caouana*, Figs. 9–15 to *Clemmys japonica* and Figs. 16–26 to *Trionyx japonicus*. The following table shows to which species different series of sections belong :—

Chelonia caouana.	*Clemmys japonica.*	*Trionyx japonicus.*
Series I	Series III	Series II
,, V	,, X	,, IV
,, IX	,, XI	,, VI
,, XIII		,, VII
,, XIV		,, VIII
		,, XII

If the reader will take the trouble to glance over these figures and series, he will, I think, justify the conclusion that the course of changes as depicted in the preceding pages has been preserved most plainly in *Chelonia caouana*, somewhat less so in *Clemmys japonica*, and is most abbreviated in *Trionyx japonicus*.

As to what light, if any, the developmental changes here brought out throw on the question of the growth in length of the embryo, I may say that so far as the examination of *normal* embryos shows, there is nothing to indicate that the anterior part of the embryo is formed by concrescence and not by intussusception. In the posterior part *i.e.*, in the part formed from the "Endwulst," the story is, however, plain, the formation of that part of the embryo by the coalescence of the blastopore lips being clearly pointed out. I shall

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not go into this question further on the present occasion, as I am engaged with certain experimental investigations on the subject, a preliminary account of which ought soon to appear in the *Anatomischer Anzeiger*.*

III. THEORETICAL CONSIDERATIONS.

I have thus far used the names, the *primitive streak*, *the primitive groove*, *the yolk-plug*, etc., more for the sake of brevity, and have not yet examined in detail the nature of the respective structures to which these names have been applied in the foregoing pages. This I propose to do in this section by comparing the course of events, which I hope, the present investigation has fully established for the group of Chelonia, with what takes place in other groups of Vertebrata.

For this purpose, I take up first—

THE ELASMOBRANCHII.

Thanks to the recent works of SCHWARZ ('89), SEDGWICK ('92) and the ZIEGLERS ('92), not to mention the older and classical memoirs of BALFOUR and others, a great deal of light has been thrown on the development of the posterior part of the embryo in this group. As what takes place in the Elasmobranchs serves as a sort of key in explaining the course of changes in Chelonia, it is necessary that the salient points of development in the first group should be given here. I quote freely from SEDGWICK (*p. 559 et seq.*) :—

"As is well known, the blastoderm attains a certain size before any trace of the embryo is visible, spreading by a uniform growth at

* Since appeared in *Anatomischer Anzeiger*. Nr. 13, XI Bd.

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all points of its circumference over the yolk. At BALFOUR'S stage A, however, the first trace of the embyo appears as a slight thickening at one point of the circumference of the blastoderm. This point is usually regarded as the hind end of the blastoderm. This is not quite correct, for it really becomes the front end of the future embryo. After the appearance of the embryonic rim the blastoderm still continues to spread over the yolk by a uniform growth of all parts of its circumference, but in the centre of the embryonic rim a slight indentation appears. This indentation shares in the uniform growth of the blastoderm edge, and advances over the yolk equally with the rest of the embryonic rim and general edge of the blastoderm. As the embryonic rim travels away from the point of its first appearance, the surface of the blastoderm so formed—that is to say, the surface of the part of the blastoderm extending between the point of first appearance of the embryonic rim and the embryonic rim at any given moment of its growth—is slightly elevated from the rest of the blastoderm, and traversed by an inconspicuous longitudinal median groove. This raised part of the blastoderm soon becomes marked off by two ridges, which in front, *i.e.* at the point which marks the site of the first appearance of the embryonic rim, are continuous with one another, while behind they are continuous with the parts of the embryonic rim which bound the indentation. These portions of the embryonic rim are more markedly swollen than the rest, and form the 'tail swellings' of BALFOUR. This elevated part of the blastoderm is the medullary plate, and the shallow groove traversing it marks the line of growth of the indentation above referred to. These points are all illustrated by my fig. 1,* which represents the embryo at a stage where the indented rim has grown back a considerable distance from the point of its first appearance. * * * *

* See the two figures copied from SEDGWICK on Pl. VII.

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"It must be clearly understood that the growth of the whole edge of the blastoderm has so far been a uniform one. The indentation in the embryonic rim advances equally (after its first establishment) with the more prominent parts of the embryonic rim called the caudal swellings. * * * *

"After a certain time the caudal swellings and the part between them begin to grow more rapidly than the adjacent portions of the edge of the blastoderm and come to project beyond the latter like a kind of tongue overhanging the yolk (Fig. 2*). * * *

"I now wish the reader to concentrate his attention upon the projecting tongue which will form the under part of the embryo. * *

"* * *—the sides of the projecting tongue become bent ventralwards and towards each other until they meet or nearly meet in the ventral middle line. Now two important structural results, which should be noted and understood follow from this bending: (*i*) the two angles formed by the junction of the edge of the blastoderm in the embryonic region with the edge of the blastoderm in the non-embryonic region—the angles, one of which is marked *a* in fig. 2,* become closely approximated ventrally beneath the embryo; and (*ii*) a space is enclosed on the ventral side of the embryo, which space is lined by endoderm, and opens ventrally to the exterior through a slit formed by the contact of the ventrally bent edges of the tongue, and dorsally into the neural canal by the slit in the medullary plate. This space is the hind gut, and the two slits which are continuous with one another round the hind end of the embryo are portions of the blastopore. By the time that the two angles marked *a* and the edges of the embryonic part of the blastoderm have come into contact ventrally, the non-embryonic edge of the blastoderm adjacent to the embryo have grown backwards over the yolk to form the bay

* See the figures copied from SEDGWICK on Pl. VII.

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mentioned by BALFOUR. The two sides of this bay, which it will be remembered are portions of the edges of the blastoderm, come to lie close together on the yolk beneath the tail of the embryo. For a little time they remain unfused, and the yolk is still freely exposed between them in a linear streak.* This slit, which is bounded by the edges of the non-embryonic part of the blastoderm of the two sides, is a part of the blastopore, and is continuous, passing along the hinder side of what will be the umbilical stalk, with the portion of the blastopore leading into the hind gut and extending along the ventral side of the tail. This last portion is * * * continuous with a dorsal portion which leads through the medullary plate into the medullary canal.

"The last part of the blastopore to be mentioned is the so-called yolk-blastopore, described by BALFOUR in the 'Elasmobranch Fishes' p. 81 (Mem. Edition, vol. iii, p. 296), and in the 'Comparative Embryology,' 1st ed., ch. iii, p. 52. The lips of this portion are continuous with the lips last mentioned as running back on the yolk parallel to one another and ventral to the tail of the embryo.

"To recapitulate: the blastopore of Elasmobranchii is at the present stage—*i.e.* the stage immediately before closing—an elongated narrow slit, slightly dilated in front, where it lies on the floor of the medullary canal (Fig. 3) and more dilated behind (Balfour's yolk-blastopore, 'Comparative Embryology, vol. ii, ch. iii, Fig. 30*b*). Between these two limits it takes the course of a reversed letter S, as shown in the adjoining woodcut,† where its lips are represented unfused.

"The anterior part, *a b*, perforates the floor of the medullary canal, and is dorsal; this is continuous round the end of the tail,

* See SCHWARZ's Fig. 16. or ZIEGLER Fig. 24, I.

† See Woodcut XII on the next page.

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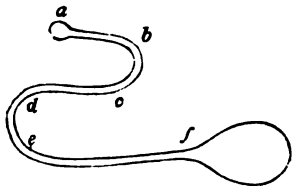
b c, with a ventral part, which extends forwards along the ventral side of the tail, *c d*, as far as the yolk-stalk, along which it passes, *d e*, to continue backwards along the yolk, *e f*, as the slit-like non-embryonic part of the blastopore, which passes behind into the more dilated and posterior part of the so-called yolk-blastopore.

Woodcut XII.



"Shortly after this stage the blastopore completely closes, excepting one point in its dorsal portion, which persists for some time as the neurenteric canal."

Thus far SEDGWICK. To make the relations brought out by him still clearer, I give here a diagrammatic figure (Woodcut XIII) constructed by myself from ZIEGLER'S figures and representing the yolk as already covered by the blastoderm excepting a small area called by BALFOUR the yolk-blastopore. The blastopore is indicated by a curved line extending from *a* to *g*, and has its different parts marked by the same letters as in the figure above given of SEDGWICK. That is, *a* is the neurenteric canal. *a b* is along the dorsal surface of the tail, *b c* around the tail end, *c d* along the median ventral line, *d e* down the yolk-stalk, *e f* the coalesced edges of the non-embryonic part of the blastopore, and finally the circular opening, *g*, exposing the yolk on the surface.

Woodcut XIII.

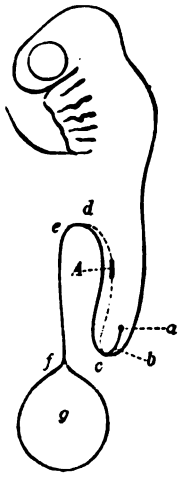


Diagram of an Elasmobranch embryo.

I must now ask the reader to compare the course of changes recounted above as taking place in the Elasmobranchs with that I

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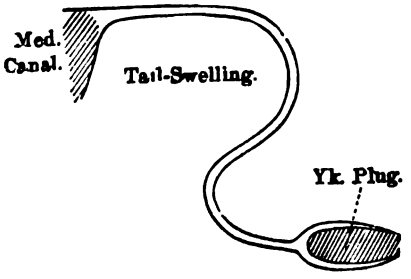
have brought out, in the course of the present article, for the group of Chelonia. The similarities are so striking and obvious that it seems hardly necessary to point them out. Let us take the *Chelonia* embryo represented in Figs. 8, 8*a*, and Series IX. There is the tail swelling enclosing within it an indifferent cellular mass in which all the layers are fused. The medullary chord ends posteriorly in this mass. The medullary canal therefore ends just in front of this mass and is at this point connected with the enteron by the neurenteric canal into which the posterior half of the original archenteron has been transformed (see Woodcut IV; also *cf. Cantrib. IV*). The hindmost end of the medullary canal or the neurenteric canal is still open dorsally, and from this opening there arises a median streak which is marked for the most part by a groove lying on it, and along which the epiblast is proliferating cells to the indifferent cell-mass within. This streak runs backwards along the dorsal surface of the tail-swelling (Series IX, Sections *g-k*), around and down its posterior tip-end to the ventral surface (Sections *k-l*), and then backwards along the median line of the non-embryonic epiblast below the tail-swelling (Sections *k-m*), until it reaches the yolk-plug at its posterior end (Section *n*). The streak diagrammatically represented is therefore as in the adjoined Woodcut. Comparing this with Woodcuts XII or XIII, we can homologise the two, point for point, and we can draw the two very important conclusions (1) that the aforesaid streak in the *Chelonia* embryo, given in Figs. 8 and 8*a* and Series IX, represents the blastopore in a condition observable in Elasmobranch embryos of such a stage as is given in Woodcut XIII; and (2) that the problematical cell-mass (Series IX, *n*) which I

Woodcut XIV.



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have called the yolk-plug is, *at this stage*, homologous with the yolk-mass peeping through the yolk-blastopore of the Elasmobranch (Woodcut XIII, *g*).

Again, if we compare Series XIII and XIV, representing the sections through the tail of somewhat more advanced chelonian embryos, with SCHWARZ's Figs. 15-22 ('**89**) or with ZIEGLER's Figs. 24, I–V, ('**92**) the similarity is very striking: some of the sections of the Elasmobranch will pass for those of Chelonia. Also, consider *how* and *where* the anus arises in Chelonia (Series XIII and XIV) and compare it with the same event in the Elasmobranch* (ZIEGLER Fig. 9*a*, *loc. cit* and BALFOUR '**78**): the two are exactly alike. In both, the proctodæum arises in a part of the primitive streak placed in the ventral median line of the tail (Woodcut XIII, *A*).

If a comparison is made of the stages before reaching that of Figs. 8 and 8*a*, we shall also find many things to strengthen the similarity. In this sense, the change of shape in the chelonian blastopore (Woodcut V., p. 14) acquires a new and deep significance. If we examine how the blastoderm comes to surround the yolk in Elasmobranchs, and notice specially its posterior edge, we shall find that at first the posterior edge has a crescentic outline with the concavity turned *anteriorly*. As the blastoderm gradually spreads over the yolk, the concavity of the crescent is gradually reduced until at one time, the posterior edge must be straight (with the median notch at the embryonic rim), and with still further progress, it acquires a crescent-shape with the concavity turned *posteriorly*. (BALFOUR. Comp. Embryol. Vol. ii. Fig. 30). After a certain time, that stretch of the posterior edge nearest the embryo on each side comes to meet the corresponding streteh of the other side in the median line, producing

* See the point *A* in Woodcut XIII.

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the streak, *e f*, in the Woodcuts XII or XIII, and removing the exposed yolk-surface some distance backwards.

How do chelonian embryos behave in this respect? The anterior lip of the blastopore representing here the posterior edge of the blastoderm* is at first crescent-shaped with its concavity turned forwards (Woodcut V. 2). It then becomes gradually straight, and with further progress acquires a crescent-shape turned posteriorly (Woodcut V. 3, 4, 5, 6). It may possibly be pushing the comparison too far to say that the median notch, noticeable in Woodcut V. 3, 4, and 5, corresponds to the indentation in the median line of the posterior edge of the blastoderm in Elasmobranchii (SEDGWICK, '92 Fig. 1=SEDWICK, Fig. 1, Pl. VII of this article, or BALFOUR, Com. Embryol. Vol. ii, Fig. 30 A); but that such a notch is present in at least some chelonian embryos, just at the stage when such ought theoretically to be visible, is significant and ought not to be left out of sight. When the enclosure of the yolk-plug by the anterior lip of the blastopore has reached a certain stage (Woodcut V. 7, or Woodcut X, *A* p. 71) the lateral lips of the blastopore press towards, and meet in, the median line, gradually removing the yolk-plug or the exposed yolk-surface backwards and producing the linear streak (the primitive streak) between the original position of the yolk-plug and its new one (Woodcut X, *B*, *C*).

In regard to the formation of the tail, there are some chronological disparities between Elasmobranchii and Chelonia. In the former group, the foundation of the tail is laid before there is any streak behind the embryo (SEDGWICK, '92, Fig. 2=SEDGWICK, Fig. 2, Pl. VII. of the present article). In the latter group, on the contrary, the

* It may be urged by some that the sickle given in some of the figures of reptilian embryos, as, for instance, in one copied from KUPFFER by HERTWIG in Fig. 65 of his *Lehrbuch* (4te Aufl. p. 95), represents the posterior edge of the blastopore, from the analogy of KOLLER's sickle in birds. But the sickle in the figure in question is formed by the accummulation of the peristomal mesoblast and has nothing to do with KOLLER's sickle.

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lips of the blastopore are fused in the median line, thus producing a streak, and the yolk-plug reaches its final position, before the tail begins to make itself perceptible (Woodcut X, *C*). This difference may be due to the fact that in Elasmobranchii, the blastoderm has to enclose *a large yolk-body* before the lips of the blastoderm can unite in a streak, while in Chelonia, only a semblance of the above process is gone through, and the anterior lip of the blastopore has to encircle only *a small rudimentary structure*—the yolk-plug. Be this as it may, the chronological disparity will not appear serious in the eye of a comparative embryologist, so long as the results are alike, as is the case in this instance.

There is another fact which is of importance in comparing the two groups. In Elasmobranchii, when the blastoderm has grown uniformly in all its circumference for some time, the caudal swellings begin to grow more rapidly than the adjacent portions of the edge of the blastoderm, and *come to project beyond the latter like a kind of tongue overhanging the yolk.* (See the quotation above from SEDGWICK, **'92**, and his Figure 2=SEDGWICK, Fig. 2, Pl. VII of the present article). In other words, the tail is formed in a manner somewhat different from the rest of the embryonic body and stands out freely as a tongue. Now, in describing the formation of the tail in Chelonia in the foregoing pages, I have repeatedly shown that the tail in that group is formed *by a new elevation or upheaval* from the surface of the blastoderm. The tail may be said to stand up from the surface of the blastoderm like a tongue. This can only be interpreted as repeating the process seen in the Elasmobranchii. The only difference lies in the anachronism pointed out above, but this, as I have said, can not be considered in comparing the two as a serious difficulty in the way.

Thus, whether we compare a stage of chelonian embryos, such as

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that given in Figs. 8 and 8*a*, and Series IX, with that of Elasmobranch embryos diagrammatically represented in Woodcuts XII or XIII, or whether we compare the course of events that take place in the two groups *before or after* such a stage is reached, the resemblances are very striking. It would be preposterous to think that all these similarities are mere coincidences. They can only be interpreted as having a deep phylogenetic significance. We may, I think, safely conclude that *the course of events described in the preceding pages as taking place in the posterior portion of the embryonic area resulting in the formation of the posterior part of the embryo in Chelonia is a repetition, in a rudimentary form, of the process affecting the homologous parts in Elasmobranchii.*

If this is really the case, and the homologies of parts are indeed those indicated in the last few pages—and I see no way of escape from this conclusion—some very important and far-reaching results follow :—

1. It has been shown both in Elasmobranchii and Chelonia that the blastopore consists at a certain stage (Woodcut X, *C*, and Woodcut XII and XIII) of three parts; (*a*) the anterior dilated part, (*b*) a groove or streak leading backwards from it and ending in (*c*) the posterior enlarged part—the yolk-blastopore. But embryologists will, I think, willingly admit that the second of these three parts in Chelonia has all the characteristics of *the primitive streak or groove* seen in other Amniota—*is in fact a primitive streak.** Hence it follows that *the primitive streak is genetically the blastopore lips or at least a part of them.*

Sedgwick (**'92**, *p. 565*) says in regard to this point :—" Balfour

* It would not be difficult to compare the primitive streak of Aves, for instance, with the above structure in Chelonia and establish their homology; but the resemblances are so obvious that I do not think it necessary to go into details.

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does not say that the two structures* are homologous; he expressly guards himself from this. He says ('Comparative Embryology,' 1st ed., vol. ii, ch. iii, p. 51). 'A linear streak [my woodcut,** *e f*] formed by the coalesced edges of the blastoderm is left connecting the embryo with the edge of the blastoderm. This streak is probably analogous to (*though not genetically related with*) the primitive streak in the Amniota' (the italics are mine). But he undoubtedly does compare the primitive streak with this linear part of the yolk-blastopore of Elasmobranchs; and he says (Comparative Embryology, vol. ii, 1st ed., ch. xi, p. 240), 'That it (primitive streak) is in later stages not continued to the edge of the blastoderm, as in Elasmobranchii, is due to its being a rudimentary organ.' The modification which I would propose to suggest in the comparison is as follows. The primitive streak of the Amniota is, as is well known, partly involved in the tail fold, and tucked under on to the ventral surface of the embryo.† It thus becomes divided into a dorsal part, at the front end of which is the neurenteric canal or its rudiment, and a ventral part. The dorsal part is in birds for some time placed in a dilated posterior part of the still open medullary groove called the *sinus rhomboidalis*. This part I would compare to the dorsal part of the blastopore shown in the same position and relations in my figs. 3 and 4. The ventral part, on the other hand, I would compare to the part of the blastopore which in Elasmobranchs runs along the ventral side of the tail to the yolk-stalk (my Woodcut *c d*);‡ while the linear part of the yolk-blastopore in Elasmobranchii (my Woodcut, *e f*) is *unrepresented or rudimentary in Aves and Amniota generally—is, in fact,*

* *I. e.*, the primitive streak of the Amniota and the linear streak on the Elasmobranch yolk.

** Copied in Woodcut XII of the present article.

† In view of the facts brought out in the present article in regard to the tail formation, I should object to the words "*tucked under*" which SEDGWICK uses.

‡ Copied in Woodcut XII of the present article.

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the rudimentary part referred to by BALFOUR *in the above quotation from the 'Comparative Embryology.'"*

The italics of the last few lines are mine. In the scheme of homology which I have sketched out above (*pp. 83–85*), the part, *e f*, in SEDGWICK'S woodcut (=Woodcut XII of the present article) which is stated by him to be rudimentary or absent in Aves and Amniota generally, is distinctly present in Chelonia as a part of the primitive streak. The fact is that neither BALFOUR nor SEDGWICK in making the above comparison ever thought that there was a rudimentary yolk-structure in the Aminota which ought to be compared with the yolk-blastopore of Elasmobranchii, and directly homologised the large yolk-mass of the Sauropsida with the yolk-mass of Elasmobranchii. This view I consider to be untenable, as will be evident from what is said under the next heading.

The identification of the primitive streak with the blastopore is no new idea, but I hope to have filled in details which make the comparison much more accurate.

2. The problematical cell-mass which stands up between the blastopore lips, and which I have all along called the *yolk-plug*, represents, in such a chelonian embryo as that of Figs. 8 and 8*a*, the large yolk-mass of the Elasmobranch egg. Hence, we must conclude that *the main body of the yolk in the chelonian egg must not be homologised with the yolk-mass of the Elasmobranch egg. It seems most reasonable to conclude that in course of phylogenetic development, the yolk which is homologous with the yolk-mass of the Elasmobranch egg, must have dwindled in size and been lost from the eggs of the ancestors of the Amniota. This yolk-mass which I may call the primary yolk mass has, however, left in some eggs, (e. g., in the chelonian egg) its rudiments in the structure known as the primitive plate and its direct derivative, the problematical cell mass behind the blastopore. In further course of*

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development, the eggs of the ancestors of the Amniota acquired for the second time a large yolk-mass, which I may call the secondary yolk-mass. It is this which we see in the Amniote eggs of the present day. The enclosure of the primary yolk-mass by the blastoderm is closely connected with the process of gastrulation and has a palingenetic character. The enclosure of the secondary yolk-mass by the blastoderm is a coenogenetic process and has nothing to do with gastrulation. Thus it becomes imperative to distinguish the meroblastic egg of the Elasmobranchii from the meroblastic egg of the Amniota. The former may be called the *primary meroblastic ovum* or *proto-meroblastic ovum*, and the latter the *secondary meroblastic ovum* or *meta-meroblastic ovum*. All comparisons of the two classes of eggs without taking the above circumstances into consideration are apt to lead us astray.

These conclusions may at first sight appear somewhat fantastic, but if the facts are really as brought out in the present article—and I have taken great pains to test their correctness and am convinced of their substantial correctness—the conclusions seem to me inevitable. The idea of the loss and acquisition of yolk in the vertebrate eggs several times in the course of phyletic development is no new one of mine. It is RABL ('89) who first formulated it and discussed it in a most masterly manner in his "Theorie des Mesoderms." While writing *Contribution IV*, the facts then in my possession convinced me of the correctness of the idea, and I discussed the question towards the end of that article. The facts brought out since and given in the present contribution appear to me to leave no choice in the matter, and furnish, to me at least, absolute proofs of the soundness of the theory.

3. It is remarkable that when once the above homology is accepted, the difficulties that have always stood in the way of homologising the icthyopsidan with the sauropsidan egg dissolve away. BALFOUR

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has long ago pointed out that "the possibility of a comparison between the Sauropsida and Elasmobranchii depends upon explanation being possible of (1) the position of the embryo *near the centre of the blastoderm*, and (2) the nature of the primitive streak." (Comparative Embryology. 1st ed., vol. ii, ch. XI, p. 236). The second of these points has already been disposed of, as the primitive streak has been shown to be identical with the blastopore lips. The other point, one which, I think, has bothered embryologists a great deal more than the second, is also cleared away. For, if the problematical cell-mass which I have called the yolk-plug represents the exposed surface of the primary yolk-mass, then *the embryo is actually formed at the edge of this yolk-mass, and not in the centre of the blastoderm. Thus a strict comparison with the Elasmobranchii in this respect is possible.*

Let us next turn our attention to—

THE AMPHIBIA.

A flood of light has lately been thrown on the process of gastrulation in Amphibia by a number of important works by ROUX, GÖTTE, MORGAN, v. ERLANGER ('89), ROBINSON and ASSHETON ('91), O. HERTWIG ('92 and '95), EYCLESHYMER ('95), and many others. A comparison of the two groups, Chelonia and Amphibia, in this respect brings out some important points interesting to us. I shall now touch on some of these points.

1. It has been shown by O. HERTWIG that the tail in the Amphibia is formed by the *rising up* of a special cell mass, and is formed in a way different from the rest of the body lying anteriorly to the tail ('92, *p. 452 et. seq.*). It, moreover, arises from a paired "*Anlage.*" This must be considered the same as the process of upheaval which I have brought to light in this article in regard to the

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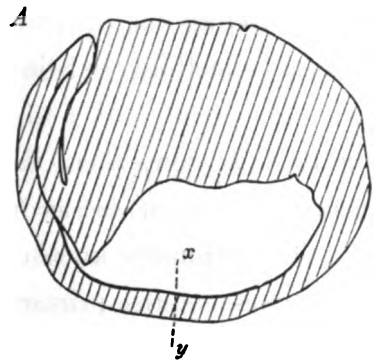
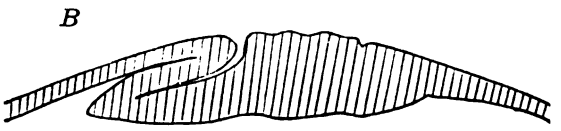
formation of the tail in Chelonia. The paired "Anlage" correspond to the two sides of the primitive streak.

(2) If we take a frog's egg, such as is represented in Woodcut XV, *A*, cut it open at the line *XY*, and spread the whole flat, we shall obtain a figure like *B*. This reminds us of the stages of Chelonia given in Woodcut IV, *C* (*p. 12*) or *Contrib. IV.* Pl. VIII, Fig. 9. It is true that in Chelonia, there is below the epiblast an under layer, which I have called the cœnogenetic hypoblast, but as this has arisen cœnogenetically around the secondary yolk-mass (*vide supra*), its absence in *B* is of no consequence. If we stretch this comparison one step farther, it makes the conclusion highly probable that the *primitive plate* or *knob* (Woodcut IV. *A*), acknowledged by WILL, MEHNERT, and myself to be present in the two layered stage of Reptilia, corresponds to *the lower, larger-celled half of the amphibian egg or in other words to the primary yolk-mass.* It might be objected that, in the frog, gastrulation commences at the boundary between the upper, and lower halves, or at the *anterior edge* of the lower half if spread flat as in Woodcut XV, *B*, while gastrulation is stated to commence in the *middle* of the primitive knob in Reptilia. If we look at Fig. 9 of my *Contrib. IV*, it will be found that the gastrulation cavity is in the middle of the primitive knob, but as I have stated above (*p. 13*), the epiblast spreads backwards to the edge of blastopore, simultaneously

Woodcut XV.



A section of a Frog's egg. After ASSHETON.



The above figure cut open at XY and spread flat.

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with, or perhaps even before, the commencement of gastrulation. In fact, I am inclined to think that the establishment of the epiblast at the future dorsal lip of the blastopore is the signal for the commencement of the invagination (*see Contrib I, p. 236*). If we also look at WILL's figures 42, 44, 48, ('**92**), or 13*a*, 13*b*, 16 ('**93**), the above objection will not hold. It seems to me that the explanation just given of the nature of the *primitive plate* or *knob* is a very satisfactory one, and has the advantage of deriving this structure from something existing already in Icthyopsida.

Woodcut XVI.

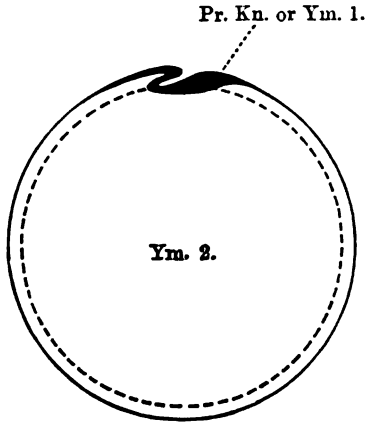

Diagram of a chelonian egg.
Pr. Kn. = Primitive knob or plate.
Ym. 1 = Primary yolk-mass.
Ym. 2 = Secondary yolk-mass.

According to this view, then, the chelonian egg may be represented as in Woodcut XVI. The primitive knob or plate (*Pr. Kn.*) represents the primary yolk-mass and is homologous with the lower larger-celled half of the amphibian ovum and with the large yolk-mass (including all the parts that do not belong to the epiblast) of Elasmobranchii. The larger yolk-mass (*Ym. 2*) of Chelonia, although developed in continuity with the primary yolk-mass, must be looked on as something newly acquired and not represented in the ovum of Elasmobranchii or of Amphibia. The hypoblast surrounding it may also be considered as cœnogenetic. The problematical cell mass, which I have all along called the yolk-plug, is directly derived from a part of the primitive plate after the process of gastrulation has taken place in the primitive plate. If the primitive plate corresponds to the lower larger-celled half of the amphibian ovum *before* gastrulation, the problematical cell-mass in such a stage as Fig. 1, Pl. I. corresponds to *the posterior*

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part of the large-celled floor of the archenteron PLUS *the yolk-plug** in the amphibian ovum, *after* gastrulation has taken place (see, for instance, *dz* and *d* in Fig. 58,** HERTWIG, *Lehrbuch, 4te. Aufl. p. 89*). At later stages, when the problematical cell-mass has receded to the edge of the embryonic area (Fig. 8 etc.), it represents less, and corresponds simply to the yolk-mass peeping through the yolk-blastopore of the Elasmobranchii. What, it corresponds to in the amphibian ovum is somewhat doubtful, as the discussion in the next paragraph will show. If the homology of different parts which I have set forth here is true, we should expect *a priori* that the primitive plate will give rise to the structures which are formed out of, or at least, in contiguity with, the lower larger-celled half of the amphibian ovum, or its homologue in Elasmobranchii, while the secondary yolk-mass ought not to be of much significance in this respect. In point of fact, the facts brought out in my *Contribution IV* fall in well with these *a priori* considerations. The conclusion which I set forth in that *Contribution* as justified by the facts brought out there are as follows :—

" *From the epiblast of the embryonic shield, the* EPIBLAST *and* ITS DERIVATIVES *of the future animal is derived. In the region of the primitive plate and its anterior enlargement are produced the* INVAGINATION-CAVITY (*the Archenteron*), *the* YOLK-PLUG, *the* CHORDA, *the* MEROBLAST (*both peristomal and gastral*), *and the* DEFINITIVE HYPOBLAST *and* ITS DERIVATIVES. *The primitive lower layer*† *forms the wall of the yolk-sac, and contributes to the future animal only in so far as some of its cells are unrecognisably incorporated with the cells of the primitive knob, when the*

* The name *Yolk-Plug* which I have applied to the problematical cell mass is accordingly not entirely happy, as the mass corresponds to something more than the yolk-plug. But as it is the name which I have used from the first *Contribution* and as it answers on the whole my purpose best, I have retained that name in this paper.

** A well-known figure, widely copied, showing the longitudinal section of a *Triton* egg after the completion of gastrulation.

† Represented by the dotted line in Woodcut XVI.

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latter spreads itself over the ventral surface of the embryonic shield." (*p. 251*). In the same *Contribution*, I have given reasons for thinking that the large yolk-mass of Chelonia (*Ym. 2*) ought to be regarded merely as a ventral appendage of the archenteron, the cavity in which has arisen because it is necessary to have the nutrient matter in the liquid form, its presence being due, therefore, solely to physiological necessity and having a comparatively insignificant morphological value.*

3. O. HERTWIG ('**95**) has recently described some abnormal embryos reared in salt solutions which remind us strongly of the chelonian or selachian embryos. For instance, in his Figs. 15 and 16, there is the tail projecting hook-like over the still exposed yolk-cells. This is like Figs. 8 and 8*a* of the present article, if we change the relative sizes of the tail and the exposed yolk. While, therefore, there can be no doubt that there is a general resemblance in this respect between the chelonian and selachian embryos on the one hand and the amphibian embryos on the other, there is a serious doubt in my own mind as to the homology of the yolk-plug in the Amphibia. It seems to be tolerably certain from the works of many recent writers that the edges of the circular yolk-plug in Amphibia, after a certain stage, press towards the median line, and meet finally in the median streak, leaving however an opening both at the anterior, and the posterior, ends. The anterior opening becomes the neurenteric canal and *the posterior the anus.* If we look at Figs. 18, 19, and 23–26 of ROBINSON and ASSHETON ('**91**) and read how the tail, with the exception of the skin, is formed from the primitive streak, how the primitive streak stretches backwards from the neurenteric canal, etc., I think, we shall be obliged to conclude that here we have the homologous processes going on as in the posterior part of Chelonia and

* I am well aware that these views are diametrically opposed to those recently advanced by LWOFF ('**94**). Future investigations must decide which of us is correct.

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Elasmobranchii. That part marked *PS* in Fig. 23 of ROBINSON and ASSHETON corresponds, on the whole, with Woodcut X, *C* of this article. So far, well and good. But it is insisted on by O. HERTWIG ('**92**, *p. 452 et. seq.*) that the anus in Amphibia lies at *the posterior end* of the blastopore. If this is really so, then what becomes in Amphibia of the part corresponding to the non-embryonic portion of the primitive streak situated behind the anus in Chelonia and Elasmobranchii—*e f g*, Woodcuts XII, XIII, and XIV? Or, is the amphibian anus an altogether different structure from the chelonian or selachian, as in the latter two groups the anus is not plainly at the posterior end of the primitive streak? It is stated by ROBINSON and ASSHETON that there are twenty sections of the primitive streak *behind the anus* in the *Rana* embryo figured in their Fig. 23. Supposing the anus to be homologous in Chelonia and Elasmobranchii on the one hand and Amphibia on the other, does that short stretch represented by twenty sections in the latter group represent the coalesced edge and the edge around the yolk-blastopore (*e f g*, Woodcuts XII–XIV) of the meroblastic egg? I leave these questions open, for I think, we have at present no means of answering them satisfactorily. At any rate, the above discussion makes it certain that the Rusconian anus in Amphibia is not homologous with the yolk-blastopore of Elasmobranchii: the former includes a great deal more than the latter, for it gives rise to the neurenteric canal, the anus, and the primitive streak between the two. The Rusconian anus of the Amphibia corresponds rather, though not exactly, to the problematical cell-mass of Chelonia in such a stage as Fig. 1, Pl. I., but not to the latter when it has moved backwards and represents only the yolk-blastopore (Fig. 8). Those who share HERTWIG'S opinion that there is no such thing as the yolk-blastopore in Elasmobranchii (*'92, p. 448 and Lehrbuch, 4te Aufl., p. 134*) may consider

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that this conclusion strengthens their position, but I venture to think that their view is not justifiable for reasons which I shall try to show in the sequel.

The above discussion makes it clear that *in the amphibian egg there is present only the primary yolk-mass, homologous with that of the Elasmobranch egg but very much smaller in quantity.* No clue has, so far as I am aware, been yet discovered within the amphibian egg itself as to whether it (1) had once a large yolk-mass like that of the Elasmobranch egg and then lost it in the course of time, or (2) represents a condition similar to that which the Elasmobranch egg must have been in before it acquired the large yolk-mass. RABL ('89) adopts the former of these alternatives. The systematic position of the Amphibia in the scale of the vertebrate phylum would incline one to do likewise, but the peculiar nature of the Rusconian yolk-plug referred to above ought to make us cautious in giving the question a final decision. It is certainly a remarkable fact that there is a greater and more precise agreement in this aspect of development between Elasmobranchii and Chelonia than between Elasmobranchii and Amphibia.

Let us next cast a glance over—

THE AVES.

When we compare the primitive streak in Birds and Chelonia, the first thing that strikes us is the great difference in the time of the first appearance of this structure in the two groups. In Birds the primitive streak is one of the first structures visible in the blastoderm, while the structure in Chelonia which I have described in the present article and called the primitive streak comes into existence only after the head, the medullary canal, the amnion, etc., have made

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considerable progress in their development. Nevertheless, few will doubt that the two are homologous, when we examine their sections, or the position of the neurenteric canal with respect to the streak, etc. Of the two, there can not be any doubt that the process seen in Chelonia has retained a more primitive character, and ought to serve as a key in unravelling various questions in regard to the primitive streak in Birds. So far as I am aware, nobody has yet discovered a structure in Aves comparable with the mass which I have called the yolk-plug; but this may be due to the fact that nobody has till now looked for it. If it exists at all, it must be in a rudimentary condition, and unless looked at in the light of what takes place in Reptilia, is most likely to be overlooked. But I believe that future investigations will throw a great deal of light on the point. The posterior end of the primitive streak in Birds is notoriously variable, and nobody has made a systematic study of it up to the present time. There is one figure in existence which makes one quite hopeful. I refer to Fig. 6, Pl. VIII of KUPFFER'S classical work ('82). It is the figure of a sparrow embryo with two mesoblastic somites. At the posterior end of the medullary canal there is figured a triangular mass which the author has marked *pr*. This can be neither more nor less than the yolk-plug at such a stage as Fig. 2 of the present article.

There is one piece of observation made on the fowl which goes flatly against my views advanced above. WHITMAN ('83) has described an abnormal form of the chick blastoderm in which the primitive streak is prolonged posteriorly to the edge of the blastoderm. One naturally concludes that this prolongation represents that coalesced edge of the non-embryonic portion of the blastopore in Elasmobranchii (*e f*, Woodcut XIII) which connects the embryo with the yolk-blastopore. This presupposes the fact that the yolk-mass of the

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Birds' egg is homologous with that of Elasmobranchii. This, as I have said, goes flatly against my idea that the yolk-mass of the Sauropsida is secondary and that which corresponds to the yolk-mass of Elasmobranchii exists in the rudimentary form. Future investigations must decide which is correct. The distinguished American morphologist himself will, however, be willing, I think, to admit that the results obtained from the painstaking observations of numerous embryos in three different species both in surface-views and sections are entitled at least to as much weight as the observation which, the author himself regrets, is confined to the surface observation of a single specimen mounted *in toto*, entirely unsupported by sections. I think it just possible that when more is known about the history of the acquisition of the *secondary yolk-mass* in the sauropsidan egg, some facts reconciling the observations of WHITMAN with those made by myself may be discovered. This, I think, is all the more possible, because I have never yet observed a distinct posterior boundary in the remnant of the primary yolk-mass in Chelonia, and the long streak on the floor of the posterior amniotic tube in *Clemmys* may, perhaps, be explained in connection with that streak observed by WHITMAN. But for the present this must remain a mere conjecture. The counter question: "If the facts brought out in the present article have not the significance assigned them here, how then are they to be explained?" would be worth a consideration in deciding the matter.

THE MAMMALIA.

In regard to this group, I shall confine myself only to the remark that the mammalian egg is acknowledged by most writers to be derived from a meroblastic egg by the gradual loss of the yolk-mass. If the views set forth above are correct, it must therefore be looked on

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as descended from the *secondary* meroblastic egg by the loss of the *secondary* yolk-mass. Hence the remnants of both the *primary*, and the *secondary*, yolk-masses ought to be found in the mammalian egg. That this is actually the case, some of the recent memoirs on the mammalian development make highly probable. I refer here only to VAN BENEDEN ('88), CARIUS ('88), and KEIBEL ('93 and '95). The first of these authors mentions a structure evidently comparable with that which I have called the yolk-plug in Chelonia, as the quotation given at the beginning of this article (*p. 3*) shows. CARIUS gives, in Fig. 7 of his article a section through HENSON'S knob of a rabbit-embryo which shows an unmistakable "yolk-plug" and is strictly comparable with Series I, *f*. of this article. KEIBEL ('95) describes a peculiar cell-mass continuous with the epiblast at the posterior end of the primitive streak in *Sus*. It would be rash to say that this cell-mass has something to do with the yolk-remnant described in this paper, but it would not be too much to say that an examination of the posterior end of the primitive streak in *Sus* in the light of the facts now brought out in Chelonia may result in the discovery of new relations not before thought of. So much seems certain that in some respects there is more agreement between Mammals and Reptiles than between Birds and Reptiles.

EXPLANATION OF DIAGRAMS I-IX GIVEN ON PL. X.

In order to facilitate the comprehension of the views set forth above, I have constructed a series of diagrams that shows the course of development in Elasmobranchii and Chelonia. They are based on the diagrams given by O. HERTWIG in his article "Urmund und Spina bifida" ('92) and in his *Lehrbuch* (*4te Aufl., pp. 135 et seq.*). These I have taken for my basis, for, being in a much read text-book,

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they are already widely understood and will make the differences which I wish to bring out easily comprehensible.

Diagrams I–IV represent the course of development in Elasmobranchii, and V–IX the same in Chelonia.

Diagrams I–III are exactly like A and B in Fig. 114, and A in Fig. 115, of HERTWIG's *Lehrbuch* (*p. 135 or* '92, *Taf XX, Figs. 1—3.*) so that they hardly need any explanation. The blastoderm limited by 1–1 in I has spread to 2–2 in II and to 4–4 in III. The embryonic rim grows in length with the growth of the blastoderm.* The edge of the blastoderm opposite to that on which the embryonic rim is placed grows much faster than the latter: hence the lines 1–1, 2–2, etc., are oblique and the exposed surface of the yolk lies in the diagrams on the right of the sphere instead of being at the pole opposite to that which the blastoderm occupies in I. In IV and IV*a*, the peculiar mode in which the blastoderm finally encloses the yolk is shown. After the tail knob has arisen, that part of the blastoderm edge nearest the tail-knob on each side comes to meet the corresponding part of the opposite side ventral to, and behind, the tail, thus removing the exposed surface of the yolk-mass some distance behind the posterior end of the embryo. The blastopore then consists of three parts, (1) the neurenteric canal (*Neurent C*, IV and IV*a*), (2) the yolk-blastopore, and (3) the S-shaped streak stretching between (1) and (2) and going round the posterior tip of the tail. The yolk exposed during all these phases is *the primary yolk-mass*. There are reasons which make me think that the whole blastoderm edge in Elasmobranchii represents the blastopore lips and that the division of it into two parts, the blastopore edge (Urmundrand) and

* This much is admitted by all, as well by those who uphold the concrescence theory as by those who oppose that theory.

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the growing edge (Umwachsungsrand), in the manner of HERTWIG, is not defensible. Of this I shall speak anon.

Diagram V, the first of the Chelonian series, is somewhat like I, but is in reality very different. In the first place, the large yolk-mass (*Ym. 2*) exposed below the limits *a–a* of the blastoderm in V is not the same as the large yolk-mass (*Ym. 1*) in I, although so much like it in appearance. *That mass in V is the secondary yolk-mass.* The primary yolk-mass or the mass corresponding to that of the Elasmobranchii is represented in a surface view by the small shaded area (*Ym. 1*) behind the line, 1–1, in the posterior part of the embryonic region. The crescentic line, 1–1, represents the anterior lip of the blastopore and corresponds to the posterior part of the blastoderm edge, 1–1, in I.

In VI, the blastoderm has grown to the edge *b–b*. The embryo has grown in length from the line 1–1 to the line 2–2, which represents the anterior lip of the blastopore *now become straight across.* As I have said before (*p. 78*), I leave for the present the question open whether the embryo has grown in length by concrescence or by intussusception. The shaded area (*Ym. 1*) behind the line 2–2 represents as before the *primary yolk-mass.*

In VII, the blastoderm has covered the secondary yolk-mass to the line *c–c*. In the embryonic region, the blastopore lips 4–4 have become horse-shoe shaped. The remnant of the primary yolk-mass (*Ym. 1*) is enclosed within the horse-shoe. The lines 1, 2, correspond to the lines similarly marked in III, and the lines 3, 4, show further growth.

In VIII, the secondary yolk-mass is covered by the blastoderm to the line *d–d*. There has taken place a great change in the blastopore. The foremost part of it has become the dorsal opening

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(*Neurent C.*) of the neurenteric canal* but behind it, the lips of the blastopore pressing from both sides towards, and meeting in, the median line have removed by their proliferation the remnant of the primary yolk-mass (*Ym. 1*) some distance behind the posterior end of the embryo, producing the primitive streak.

In IX, the blastoderm has covered the secondary yolk-mass (*Ym. 2*) to the line *e-e*. In the region of the primitive streak, the tail-swelling has arisen, changing the hitherto straight primitive streak into a S-shaped curve (*IXa*). The upper part of this figure corresponds to the whole of the egg in IV. *Ym. 1* is in both cases the yolk-blastopore. Between this (*Ym. 1*) and the neurenteric canal (*Neurent. C.*) there is in both figures a S-shaped streak. The similarity is too striking to need any further explanation. Note that the final enclosure of the secondary yolk-mass takes place at the pole *opposite* that which the blastoderm occupies in V, instead of being at some spot *oblique* to the original axis as in the case of the primary yolk-mass in IV. My views beautifully account for this difference.

Thus far, the explanation of the diagrams.

In that series of diagrams given by HERTWIG, the edge of the blastoderm is divided into the growing edge (Umwachsungsrand) and the blastopore edge (Urmundrand) in both Elasmobranchii, and Sauropsida. He altogether denies the existence of such a thing as the yolk-blastopore. He says ('92, *p. 448*) :—BALFOUR hat diesem Ring den Namen Dottterblastoporus beigelegt und in ihm einen Theil des Urmunds erblickt. Wie schon früher gesagt, halte ich diese in der Literatur weit verbreitete Ansicht für eine irrige. Denn erstens liegt am sogenannten " Dotterblastoporus " überhaupt keine Oeffnung vor,

* It is often stated that the neurenteric canal is a part of the blastopore. Strictly speaking, this is not true, for the neurenteric canal is a transformation of the posterior part of the archenteron (*Contrib. IV.*). The *dorsal opening* of the neurenteric canal is however, a part of the blastopore.

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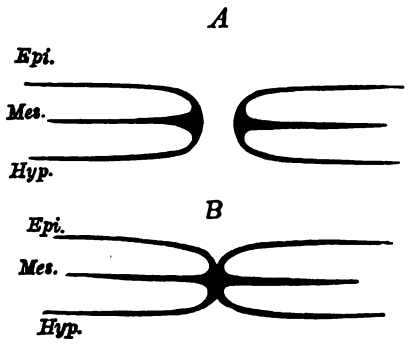
durch welche man in einen Hohlraum gelangt, wie es am Urmundrand, soweit er noch nicht verwachsen ist, der Fall ist. Durch den Umwachsungsring kommt man in die Dottermasse, die wir uns auch durch einen Haufen von Dotterzellen in welche sich der Umwachsungsrand verliert, ersetzt denken könnten." Also, in his *Lehrbuch* (*4te aufl. p. 134*), HERTWIG says: "Während am Urmund Einstülpung bereits vorhandener Zellen stattfindet, erfolgt am Keimscheibenrand Neubildung von Zellen, dadurch Vergrösserung des Randtheils und Umwachsung des Dotters. Ich schlage daher für ihn den Namen Umwachsungsrand der Dotterkugel vor. Von einer besonderen Oeffnung oder einem Dotterblastoporus kann nicht die Rede sein * * *."

It seems to me that HERTWIG was led to this conclusion, because at the time he wrote, there was no fact which made him think that *the yolk of the Elasmobranch egg is not homologous with that of the Sauropsida.* So, looking at them as alike—as indeed all the writers except RABL have hitherto done—he took it for granted that what was observed at the growing edge of the sauropsidan blastoderm takes place, also at the growing edge of the blastoderm in Elasmobranchs. But I believe, it is possible to show, that such is not the case. Writers on the Elasmobranch development show that the peristomal meroblast is proliferated from the edge of the blastoderm, where the epiblast is reflected and becomes continuous with the hypoblast. The figures given by RABL ('89), SCHWALZ ('89), and ZIEGLER ('92) all go to show this. ZIEGLER'S figure 5 shows how this mesoderm formation goes round nearly the whole edge of the blasto-

Woodcut XVII.



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derm. Woodcut XVII, *A* shows two of these edges approaching each other. Either of these edges will hardly answer the description of a growing edge by HERTWIG, and when these meet (Woodcut XVII, *B*) the line of junction has all the characteristics of a primitive streak. So that there can be no question that the blastoderm edge of the Elasmobranch at earlier stages may certainly be considered the blastopore lips (Urmundrand). When the blastoderm of the Elasmobranch has surrounded the yolk, as in Woodcuts XII or XIII (*p. 91*), what structure the meeting edges, *e f*, and the edge round the yolk, *g*, have is a question which unfortunately I can not decide by appeal to actual sections or to figures given by authors, for I do not possess the former and I do not know of the existence of the latter. The nearest approach to it is in Figs. 24, I of ZIEGLER and Fig. 16, of SCHWARZ. Both represent that region ventral to the tail where the blastoderm edges are to meet in a linear streak, but in the figures, there is still the yolk exposed between the two edges. As far as I understand these figures, the epiblast is reflected and becomes continuous wtih the hypoblast, but the proliferation of the mesoblast is already given up. I believe, the latter fact may be explained by the consideration that the blastoderm has been so long in encircling the yolk that the mesoblast formation has been completed, and the middle layer has lost its connection with the primary layers. The blastoderm edge may therefore still be considered morphologically as the blastopore lips. At any rate, it seems to me to have more the characters of blastopore edge than those of a simple growing edge, especially when we remember what they were in earlier stages. Moreover, the parts in Chelonia, which I trust to have proved to be homologous with the blastoderm edge of Elasmobranchii, have unmistakably the characters of the blastopore lips.

In regard to the edge of the blastoderm in Sauropsida, all that

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HERTWIG says in regard to the simple growing edge (Umwachsungsrand) applies to it. Unfortunately I have no observation of my own, but the work of H. VIRCHOW ('92) proves this abundantly.

This goes to prove that the edge of the blastoderm in Elasmobranchii is the *blastopore edge* and not the growing edge, while the edge of the blastoderm in Sauropsida is the *growing edge* and not the blastopore edge, which is found in their eggs in that part surrounding a small remnant of the primary yolk-mass situated near the embryo.

The objection which HERTWIG raises against the idea of a yolk-blastopore is thus not insurmountable.

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THE CLASSIFICATION OF VERTEBRATE EGGS.

The considerations set forth in the preceding pages make necessary a reclassification of the eggs of Vertebrata. I append below a table of classification such as I conceive it :—

I. Primary Type:—

Archi-Holoblastic :—*Amphioxus.*

Proto-Holoblastic :—Eggs that have never acquired a large yolk-mass and hence have retained the primary condition nearest to Amphioxus. *Cyclostomi.*

Proto-Meroblastic :—(*a*) Eggs that have acquired a large primary yolk-mass. *Elasmobranchii,*

(*b*) or eggs that having lost the large primary yolk-mass have at least retained the meroblastic mode of segmentation. *Teleostei.*

Meso-Holoblastic :—Eggs that had at one time acquired a large yolk-mass but have lost it, returning to the holoblastic condition. *Amphibia* (*?*)

II. Secondary Type:—

Meta-Meroblastic :—Eggs that had at one time acquired a large primary yolk-mass, had then lost it, and now have acquired a large yolk-mass for the second time. *Reptilia, Aves.*

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Meta-Holoblastic:—Eggs that have run a course like the meta-meroblastic eggs, but have again lost the secondary yolk-mass and returned to the holoblastic mode of segmentation. *Mammalia.*

Imperial University,
Tōkyō, Japan.
February 10, 1896.

In conclusion I wish to express my thanks to Prof. Edward Divers, F.R.S., for kindly looking through the proof-sheets.

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POSTSCRIPT.

At the end of January of the present year, I received from Dr. LUDWIG WILL a copy of his article entitled "*Die Anlage der Keimblätter bei der Eidechse* (*Lacerta*)."* As the foregoing article had then nearly taken its final shape, it was not possible to take Dr. WILL'S new article into consideration in the body of the article. I have for this reason decided to add a postscript in order to put together some remarks on Dr. WILL'S paper.

While WILL gave in two former papers ('92 & '93) the course of development in the early stages of *Platydactylus* and *Cistudo*, he has in this paper given the results of his investigations on the same phases in the embryology of *Lacerta*. His observations begin from the appearance of the primitive plate and end with the formation of the mesoblast and the primitive groove. As we are for the present most interested in the last part, we shall confine ourselves only to a few remarks on the earlier phases.

As regards the characters of the primitive plate, and the process of gastrulation WILL has obtained on the whole the same results in *Lacerta* that he obtained before in *Platydactylus* and *Cistudo*. For instance, he insists again on the sharp limits between the epiblast and primitive plate (*y*, *y* in A, Woodcut II of the foregoing article). On some points, however, he gives different results. The following are some of these points:—

(*a*). While insisting in general on a distinct boundary between the primitive plate and the epiblast, WILL confesses that he was often unable to find such a limit on the posterior end of the primitive plate.

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* *Zoologischer Jahrbücher, Abth. f. Anat. u. Ont. der Thiere. IX Bd. 1895.*

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He also says: "Die Möglichkeit, bei den Reptilien die Primitivplatte scharf von dem umgebenden Ektoderm abzugrenzen, dient nur noch zur Unterstützung der angeführten Beweisgrunde; das Fehlen einen solchen Grenze würde, wie wir gleich sehen werden, an der Auffassung nichts ändern können" (*p. 11*). He thus allows the possibility of the absence of such a limit.

(*b*). In regard to the shape of the primitive plate at its earliest phase, WILL made it in *Platydactylus* and *Cistudo* sickle-like afterwards changing to a more rounded form (Woodcut I, p. 8 of the foregoing article). In regard to *Lacerta* he says: "Da demnach die Länge der Primitivplatte der Breite derselben fast gleichkommt, so kann man bei der Eidechse nicht von einer so ausprägten Sickelform derselben sprechen wie beim Gecko und Schildkröte." (*p. 23*).

(*c*) As a corollary of the above fact, the first shape of the invagination cavity in *Lacerta* is rounded, different from *Platydactylus* and *Cistudo* (see Woodcut I of the foregoing article) in which it is either sickle-shaped or rounded (*p. 33*). [Compare my remarks on p. 14 of the foregoing article]

(*d*). In *Platydactylus* and *Cistudo*, WILL insisted on it that the invagination-cavity—the archenteron—attains an extraordinary size, and in *Cistudo* at least becomes both in length and breadth exactly co-extensive with the embryonic shield before it breaks through below. In *Lacerta* he finds that the invagination-cavity does not become nearly as large. (*p. 41*).

(*e*). The floor of the invagination-cavity is, in *Lacerta*, formed by many layers of cells. "Die untere Urdarmwand weicht jedoch in dem mir vorliegenden Präparat in so fern von dem Verhalten bei den beiden andern erwähnten Reptilien-vertretern ab, als sie bei der Eidechse fast bis in die Spitze mehrsichtig ist, während bei jenen der

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weitaus grösste Theil der untern Urdarmwand aus einen einsichtigen Plattenepithel bestand." (*p. 42*).

(*f*). When the invagination cavity breaks through below in *Platydactylus* and *Cistudo*, the floor of the cavity showed, according to WILL, extraordinary net-like appearances ('93; Figs. 6*b*, 7*b*, and 8*b*, '92 Fig. 17). In *Lacerta*, (at least in the specimen which WILL had) the invagination-cavity breaks through *at only one place.*

(*g*). The " Kupffer's Gang ", which I have called the blastoporic passage in the foregoing article, does not become closed at any time in *Lacerta*, but becomes directly transformed into the *neurenteric canal.*

On most of these points, I pointed out categorically in my *Contribution IV*, *Postscript*, the differences between the results which WILL obtained in *Platydactylus* and *Cistudo* and those which I obtained in *Chelonia. It is to be noted that the results which* WILL *now gives for Lacerta approach on most of these points nearer mine for Chelonia than those he brought out for the two species which he studied before.*

After the breaking through of the invagination cavity, the dorsal wall of the cavity is said by WILL to spread itself still further in breadth. " Wenn demnach bei der Eidechse, anders als beim Gecko, der Urdarm noch vor Erreichnung seiner definitiven Länge und Breite noch unten durchbricht, so wächst doch die dorsale Urdarmwand selbstständig genau in derselben Weise weiter wie vor dem Durchbruch." (*p. 54*). The growth is probably the same thing as the spreading of the primitive knob which I described in my *Contribution IV*. On p. 250 of that paper, I said, summing up the process. " The primitive knob which was gradually spreading itself over the ventral surface of the embryonic shield before the breaking through of the invagination cavity *continues to do so after that event.* It spreads from the spot where the invagination-cavity first broke through away

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towards the periphery of the shield." For details, the reader is referred to that paper.

I now come to that part of WILL'S paper which at present concerns us most—*viz.*, to that part which treats of the formation and nature of the primitive streak and groove. I shall not here go into the theory which he advances and illustrates by the figure L (*p. 61*), for at the present state of our knowledge a theory that makes the primitive streak grow forwards from the posterior end is in my opinion not tenable. And if I understand WILL aright, his theory has such a characteristic. DUVAL'S observations alone would be enough to contradict such a theory, not to speak of many facts which have been brought forward in support of the concrescence-theory. I shall therefore here rather confine myself to the account which WILL gives of the changes observable on the surface and in sections. And in this I confess to a sense of disappointment. A glance over his figures shows that he had before him some significant stages very much resembling those I give in this paper. I refer more especially to those on his Taf. 3. But the explanation he gives of their nature seems to me to be wholly inadequate. He simply restates his views, just as I have given them in the earlier part of the foregoing article (*pp. 8–10*), and explains everything by them. Thus, in his Figs. 22–27, in which a narrow groove goes backwards from the posterior end of the medullary groove—very much like my Figs. 4 *et seq.* of the foregoing article, if we take away the yolk-plug at the posterior end of the primitive groove—he considers the primitive groove produced by the simple coming together of the blastopore lips, *after the sinking below of the yolk-plug*. Moreover, he considers that the primitive streak is posteriorly entirely surrounded by the medullary folds (*p. 70*), and therefore all the changes, he describes, in the primitive streak takes place presumably within the medullary folds. *He has therefore entirely*

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failed to notice either the posterior elongation of the primitive streak and groove or the backward movement of the yolk-plug. It would be rash in me to say how the course of events shapes itself in *Lacerta* in regard to these points, but that groove behind the medullary canal seen in WILL'S Figs. 22, 23, 24, 25, 26*a*, and 27*a* could only have been produced by a process similar in nature to that which I have described in the foregoing article for the three species of Chelonia. This conviction is all the more strengthened, because in his Fig. 25 a structure which strongly reminds one of such a yolk-plug as I have described in the foregoing article is visible at the posterior end of the primitive groove. Also in his Fig. 21, the posterior ends of the medullary folds embrace between themselves a wedge-shaped structure which can be nothing else than the yolk-plug. WILL himself explains this by saying that the anterior halves of the primitive plate have come together faster than the posterior halves. I believe that if the posterior end of the primitive streak in such *Lacerta* embryos as are represented in WILL'S Figs 22–27*a* is critically examined, there will be found a yolk-plug, which, if not visible in surface-views, ought to be recognisable in sections. As I have said in the foregoing article, the yolk-plug at such stages is very difficult to detect. In some *Trionyx* embryos, I could detect it only by turning them round and round in the direct sunlight or on clear bright days, when the light was reflected at a certain angle. It is possible that if I had not had comparatively clear pictures of *Chelonia* and *Clemmys* for comparison, I should never have suspected the existence of the yolk-plug in *Trionyx* which would thus have remained unknown to me. It seems to me that this consideration accounts for WILL'S failure to recognise the yolk-plug. As the posterior elongation of the *primitive groove* is, however, well marked in *Lacerta*, as his figures show, the yolk-plug may perhaps be recognisable with comparative ease, if once one looked for it. What I am inclined

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to suppose to be the course of development in *Lacerta*, looking at WILL'S figures, is as follows :—After such a stage as his Fig. 16 is reached (in which WILL is fortunate in having obtained an embryo with the yolk-plug with its posterior boundary sharply defined), the blastopore lips press towards the median line, and at the same time, the posterior ends of the medullary folds reach the yolk-plug and embrace it between them. His Fig. 21 is probably at or near this stage. After this, the primitive streak and groove are posteriorly elongated, and *pari passu* with it the yolk-plug which is always at the posterior end of the groove moves backwards. WILL'S Figs. 22–27*a* show different stages in the progress of this event.

While I can not thus accept WILL'S views in regard to the closure of the blastopore lips, I find his figures very clear and instructive. They present a series of pictures which fall in well with the course of events I have described as occurring in the three species I have studied.

Feb. 14, 1896.

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PLATE I.

(Chelonia caouana.)

FIG. 1.—Dorsal view of an embryo of *Chelonia caouana* taken out 9½ days after its deposition. The anterior amniotic fold is just beginning to rise, but has not yet covered the head. The dark median streak is caused chiefly by the notochord. The medullary folds have arisen in the neck-region. The horse-shoe shaped opening in the posterior part is the blastopore. For further particulars see p. 17. Sections given in Series I (Pl. IV). Zeiss. *aa* × 2. (B 11*b*)

FIG. 1*a*.—Ventral view of the same embryo. The prominent transverse fold in the anterior part is the head. The ventral opening of the blastoporic passage is quite large. *aa* × 2.

FIG. 2.—Dorsal view of an embryo of *Chelonia caouana* taken out 10 days after its deposition. The amniotic fold has now covered the lateral parts of the head. The medullary folds have now arisen but are not closed. See p. 18. *aa* × 2. (A 3*a*)

FIG. 3.—Dorsal view of an embryo of *Chelonia caouana*, taken out 6½ days after its deposition. The amnion has covered the anterior half of the embryo. The medullary canal is still open at the posterior end. See p. 20. *aa* × 2. (C 7*a*)

FIG. 4.—Dorsal view of an embryo of *Chelonia caouana* taken out 6½ days after its deposition. Two wing-like expansions on each side of the anterior dorsal region are the extra-embryonic cœlomic cavities. The amnion covers only the anterior half. The medullary canal is still open at the posterior darkened portion. See p. 21. *aa* × 2. (C 7*b*)

FIG. 5.—Dorsal view of an embryo of *Chelonia caouana* with 9 mesoblastic somites taken out 9½ days after its deposition. The amnion has extended itself over nearly the whole of the embryo, but leaves a small posterior portion still exposed. *Pari passu* with the amnion, the extra-embryonic cœlomic cavities have also enlarged themselves. They come near together in

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the median line over the mid-dorsal region, but diverge both anteriorly and posteriorly. The medullary canal is still open at the posterior darkened place. See p. 21. Sections given in Series V (Pl. V). *aa* × 2. (B 11 ν)

FIG. 5*b*.—Dorsal view of the posterior part of the same embryo under greater magnification. *AA* × 2.

FIG. 6.—Dorsal view of the posterior part of another embryo taken out from the same deposit at the same time as that represented in Fig. 5. See p. 22. *AA* × 2. (B 11*α*)

FIG. 7.—Dorsal view of an embryo of *Chelonia caouana* taken out 14 days after its deposition. The amnion leaves only the small posterior portion of the embryo exposed. The extra-embryonic cœlomic cavities touch each other in the median line over the anterior two thirds of the dorsal region, but diverge towards the posterior portion. See p. 23. *aa* × 2. (A 8 *ι*)

FIG. 8.—Dorsal view of an embryo of *Chelonia caouana*, with about 16 mesoblastic somites, taken out 10½ days after its deposition. The amnion has not only covered the embryo, but extended itself as a tube some distance behind it. The extra-embryonic cœlomic cavities diverge from each other towards the posterior end of the embryo. See p. 24. Sections in Series IX (Pl. VII). *aa* × 2. (B 12 *ι*)

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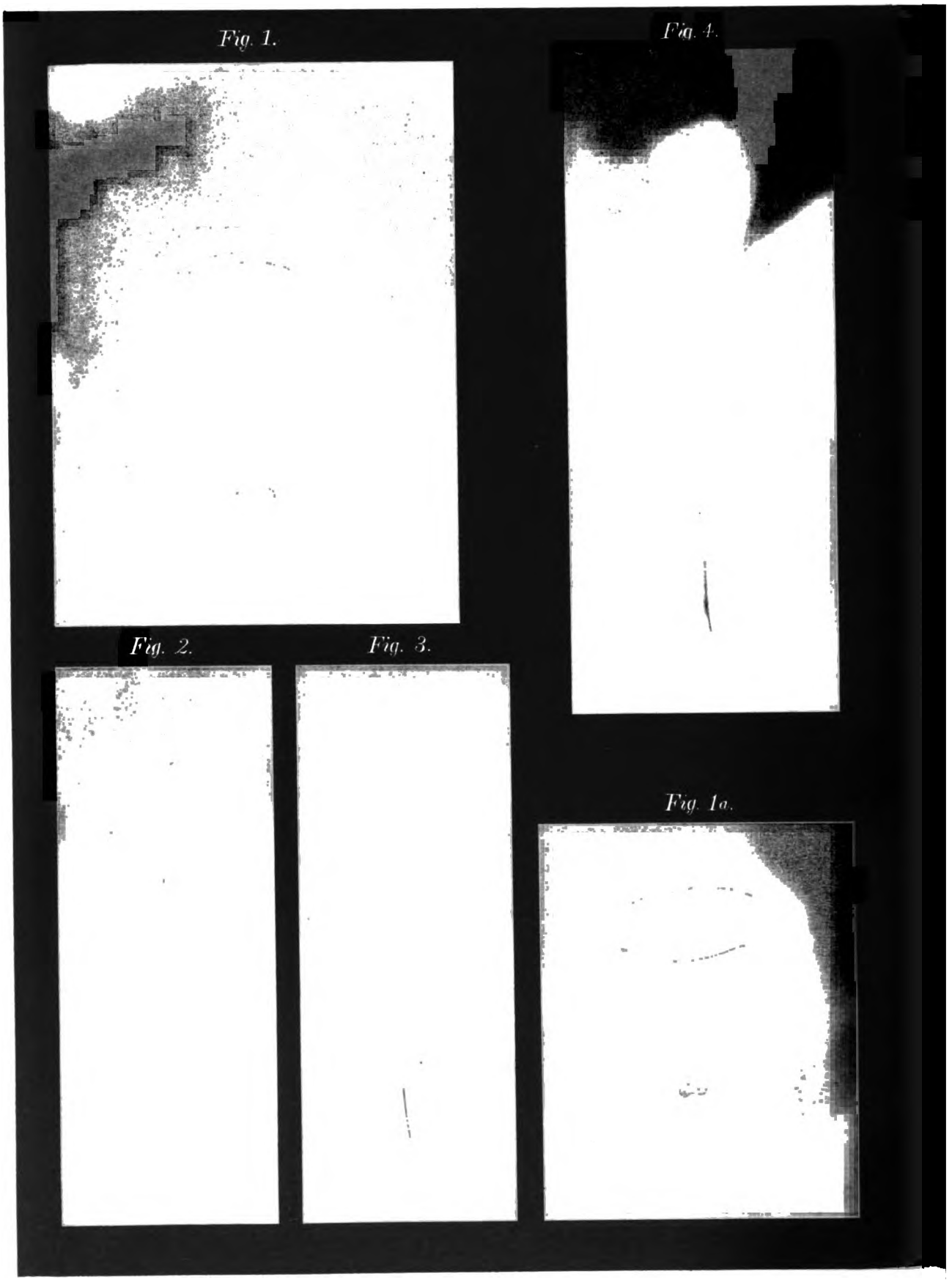

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Fig. 5.

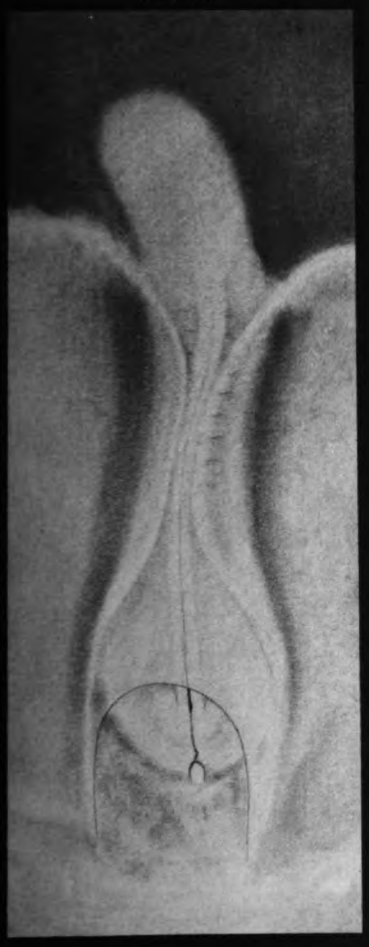

Fig. 7.

Fig. 8.

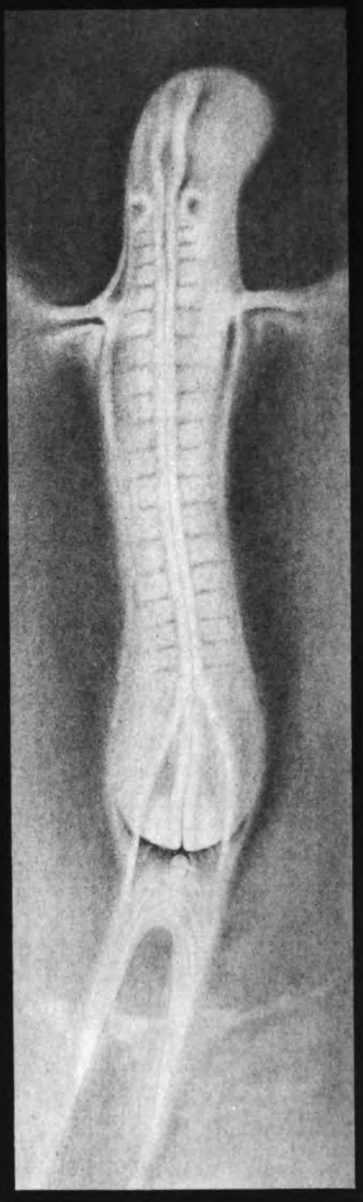

Fig. 5b.

Fig. 6.

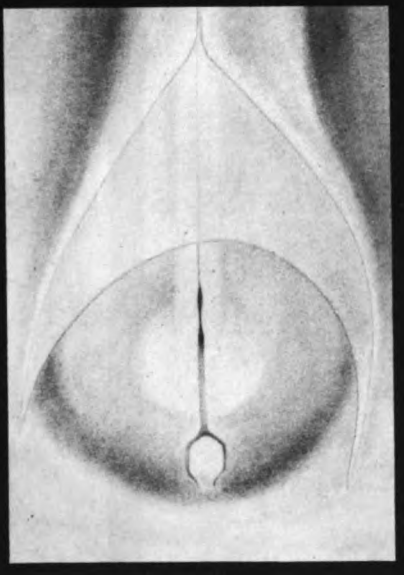

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PLATE II.

(Chelonia caouana.)

FIG. 5*a*.—Ventral view of the embryo of *Chelonia caouana* represented in Fig. 5. *aa* × 2 (B 11 ٨)

FIG. 8*a*.—Ventral view of the embryo of *Chelonia caouana* represented in Fig. 8. *aa* × 2. (B 12 ٤)

(Clemmys japonica.)

FIG. 9.—Dorsal view of an embryo of *Clemmys japonica* taken out 8 days after it[8] deposition. The amnion is just arising, and the medullary canal is open throughout. See p. 25. *aa* × 2. (XXXIV)

FIG. 10.—Dorsal view of an embryo of *Clemmys japonica* with 5 mesoblastic somites, taken out 4½ days after its deposition. The amnion covers a little less than half the length of the embryo. See p. 25. Sections in Series III. (Pl IV). *aa* × 2. (XXXIX)

FIG. 11.—Dorsal view of the posterior part of an embryo of *Clemmys japonica* with 6–7 mesoblastic somites, taken out 5½ days after its deposition. The amnion leaves only a small posterior portion of the embryo still exposed. The medullary canal is still open at the posterior darkened portion. The two white lines which diverge posteriorly from the mid-dorsal region are the internal limits of the extra-embryonic cavities. See p. 25. *AA* × 2. (L ⊕)

FIG. 12.—Dorsal view of the posterior part of an embryo of *Clemmys japonica* with 6–7 mesoblastic somites, taken out 4 days after its deposition. The amnion leaves the posterior part still exposed. The medullary canal is now entirely closed. The white diverging lines on the mid-dorsal region are the internal limits of the extra-embryonic cœlomic cavities. See p. 26. *AA* × 2. (LV)

FIG. 13.—Dorsal view of the posterior part of an embryo of *Clemmys japonica* with 6–7 mesoblastic somites, taken out 6 days after its deposition. The

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medullary canal entirely closed. The amnion and the cœlomic cavities as in Fig. 12. See p. 26. $AA \times 2$. (XXXVIIIα)

FIG. 14.—Dorsal view of the posterior part of an embryo of *Clemmys japonica* with 8–9 mesoblastic somites, taken out 6½ days after its deposition. See p. 27. $AA \times 2$. (LI β)

FIG. 15.—Dorsal view of an embryo of *Clemmys japonica* with about 13 mesoblastic somites, take out 8 days after its deposition. The amnion is produced in an elongated posterior tube. The internal limits of the extra-embryonic cœlomic cavities diverge at the posterior end ef the embryo proper, but the cavities themselves are now very large. See p. 27. Sections Series XI (Pl. IX). $aa \times 2$. (XXXXIII ι)

(Trionyx japonicus).

FIG. 24.—Dorsal view of the posterior portion of an embryo of *Trionyx japonicus*, taken out 7½ days after its deposition. The internal limits of the extra-embryonic cœlomic cavities diverge posteriorly but the cavities themselves go clearly around the posterior limit of the embryonic area. See p. 29. Sections Series VII (Pl. VI). $AA \times 2$. (155 □)

FIG. 25*a*—Ventral view of the posterior portion of an embryo of *Trionyx japonicus* whose dorsal view is given in Fig. 25 (Pl. III), taken out 9½ days after its deposition. $AA \times 2$. (151 ⊞)

FIG. 26*a*.—Ventral view of the posterior portion of an embryo of *Trionyx japonicus*, whose dorsal view is given in Fig. 26 (Pl. III), taken out 10½ days after its deposition. $AA \times 2$. (157 ⊞)

(By an unfortunate mistake, this is marked Fig. 26 on the plate).

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Fig. 8a.

Fig. 5a.

Fig. 13.

Fig. 9.

Fig. 10.

Fig. 11.

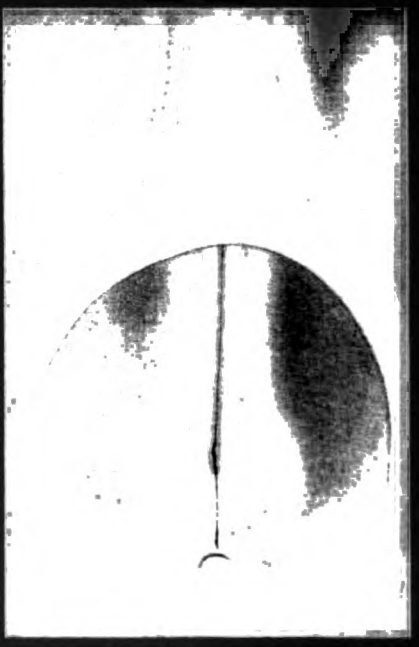

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Fig. 14.

Fig. 15.

Fig. 26 a.

Fig. 25 a.

Fig. 12.

Fig. 24.

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PLATE III.

(Trionyx japonicus).

Fig. 16.—Dorsal view of an embryo of *Trionix japonicus*, taken out 8½ days after its deposition. The amnion is just beginning to cover the head. The medullary folds arisen except in the posterior portion, and still wide open. See p. 17. *AA* × 2. (126*a*)

Fig. 16*a*—Ventral view of the same embryo. *AA* × 2.

Fig. 17.—Dorsal view of an embryo of *Trionyx japonicus*, taken out 4 days after its deposition. The amnion has covered a part of the head. The medullary folds wide open, showing the *sinus rhomboidalis* in the posterior portion. For further particulars see p. 17. *AA* × 2. (136*α*)

Fig. 18.—Dorsal view of an embryo of *Trionyx japonicus*, taken out 4 days after its deposition. The head is now covered by the amnion. The medullary canal is still wide open. See p. 17. Sections Series II (Pl. IV). *AA* × 2. (136*β*)

Fig. 19.—Dorsal view of an embryo of *Trionyx japonicus* with 4 mesoblastic somites, taken out 5½ days after its deposition. The amnion covers the head and neck region. The extra-embryonic cœlomic cavities appear wing-like on both sides of the anterior dorsal region. The medullary folds apposed but not fused. See p. 28. Sections Series IV (Pl. IV). *AA* × 2. (128*a*)

Fig. 20.—Dorsal view of the posterior two-thirds of an embryo of *Trionyx japonicus* with 6 mesoblastic somites, taken out 6½ days after its deposition. The amnion still leaves the larger part of the dorsal region exposed. The medullary canal closed. See p. 29. *AA* × 2. (135)

Fig. 21.—Dorsal view of the posterior half of an embryo of *Trionyx japonicus* with 7–8 mesoblastic somites, taken out 7½ days after its deposition. The amnion leaves the posterior half of the dorsal region exposed. The medullary canal closed. There is a slight artificial rent in the posterior edge of the amnion, a little to the left of the median line. The white lines marking the internal limits of the extra-embryonic cœlomic cavities meet over the mid-dorsal region but diverge posteriorly. See p. 29. *AA* × 2. (141 田)

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FIG. 22.—Dorsal view of the posterior half of an embryo of *Trionyx japonicus* with about 10 mesoblastic somites, taken out 8½ days after its deposition. Similar to *Contrib. II*, Pl. III, Figs. 19 and 19*a*. The amnion leaves only a small posterior portion exposed. The white lines which mark the internal limits of the extra-embryonic cœlomic cavities are still wide apart even in the mid-dorsal region. They diverge posteriorly. See p. 29. Sections Series VI (Pl. V). *AA* × 2. (143 ⊞)

FIG. 23—Dorsal view of the posterior half of an embryo of *Trionyx japonicus* taken out 8½ days after its deposition. See p. 29. *AA* × 2. (142 ⌂)

FIG. 25—Dorsal view of the posterior portion of an embryo of *Trionyx japonicus* with about 13 mesoblastic somites, taken out 9½ days after its deposition. The white lines making the internal limits of the extra-embryonic cœlomic cavities diverge over the posterior portion of the embryo, but the cavities have now gone around the posterior outline of the embryonic region and fused into one behind the embryo. See p. 30. Sections Series VIII. (Pl. VI). *AA* × 2. (151 ⊞)

FIG. 26.—Dorsal view of the posterior portion of an embryo of *Trionyx japonicus* with about 15 mesoblastic somites, taken out 10½ days after its deposition. The amnion has covered the entire embryo-body and extended just one step farther posteriorly. The extra-embryonic cœlomic cavities have not yet extended into the posterior part of the amnion, but diverge at that point. They go clear around, and unite behind, the embryonic region. See p. 30. Sections Series XII. (Pl. VIII). *AA* × 2. (157 ⊞)

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Fig. 16.

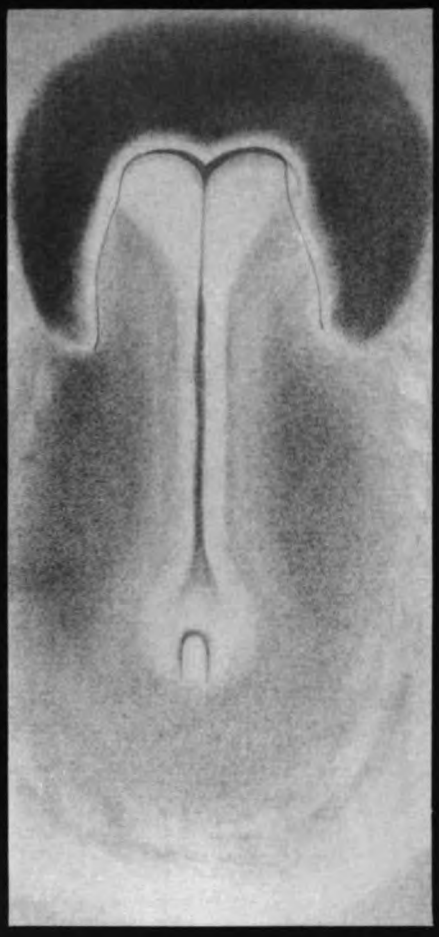

Fig. 16a.

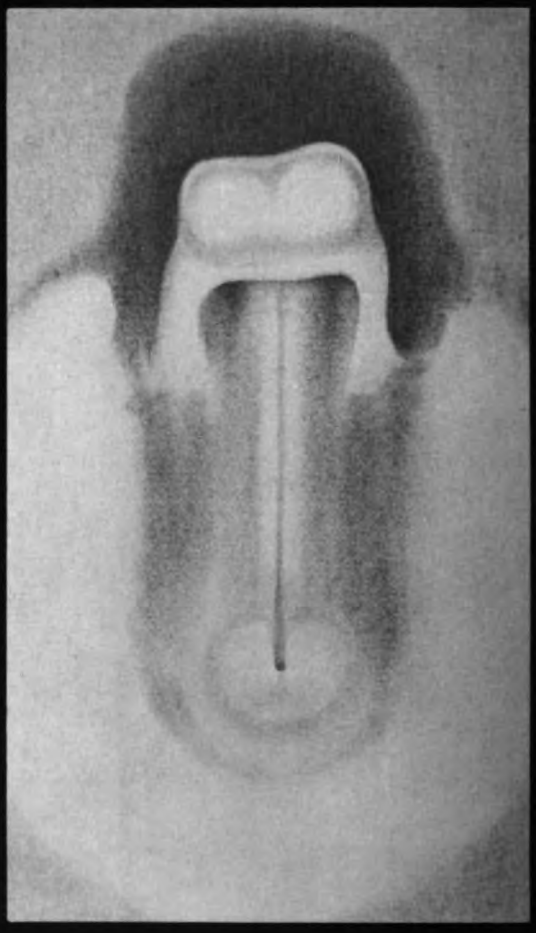

Fig. 21.

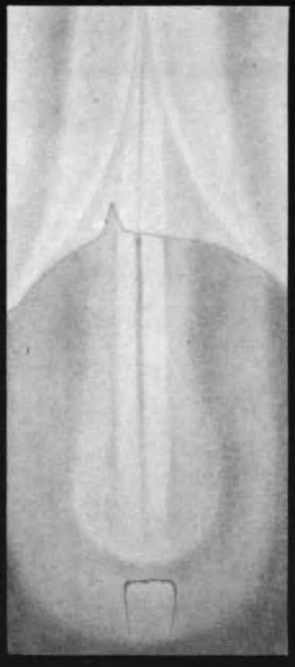

Fig. 20.

Fig. 22.

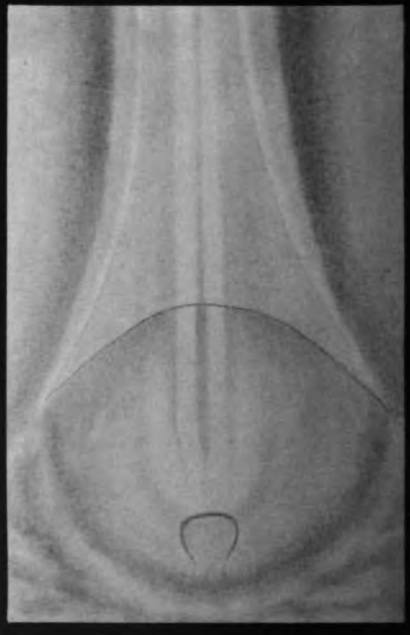

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Fig. 17.

Fig. 18.

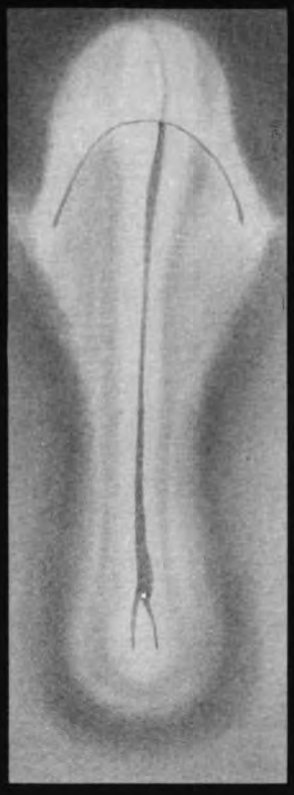

Fig. 19.

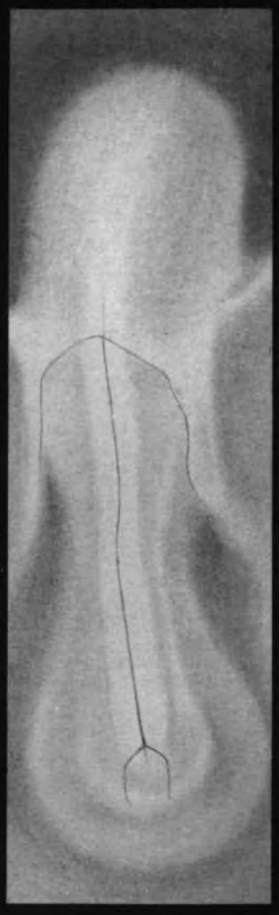

Fig. 23.

Fig. 25.

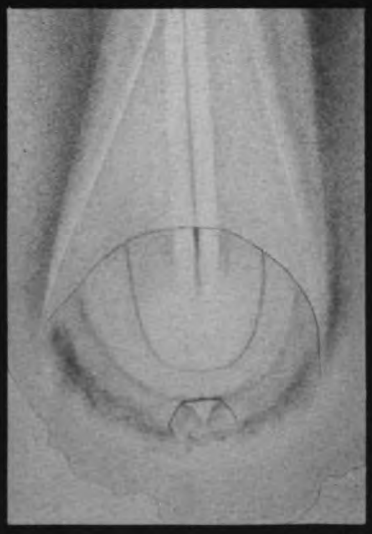

Fig. 26.

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PLATE IV.

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Plate IV.

Arabic numerals placed between every two sections indicate the number of sections that intervene between those two (exclusive).
Certain marks on the left of each section are for the identification of the sections and do not concern the reader.

Series I. *a–g.*—Sections through an embryo of *Chelonia caouana*, with no mesoblastic somites; taken out 9½ days after deposition. Surface views given in Figs. 1 and 1*a*, Pl. I. For detailed description, see p. 33. *CC* × 2.

(B 11*b*)

Series II. *a–h.*—Sections through an embryo of *Trionyx japonicus* with 2–3 mesoblastic somites, taken out 4 days after deposition. Surface view given in Fig. 18, Pl, III. For detailed description, see p. 36. *CC* × 2. (136 *β*)

Series III. *a–m.*—Sections through an embryo of *Clemmys japonica* with 5 mesoblastic somites, taken out 4½ days after deposition. Surface view given in Fig. 10, Pl. II., (=*Contrib.* II, Pl. I, Figs. 2 and 2*a*). For detailed description see p. 39. *CC* × 2. (XXXXIX)

Series IV. *a–l.*—Sections through an embryo of *Trionyx japonicus* with 4 mesoblastic somites, taken out 5½ days after deposition. Surface view in Fig. 19, Pl. III. (and also in *Contrib. II*, Pl. III., Figs. 17 and 17*a*.) For detailed description see p. 42. *CC* × 2. (128*a*)

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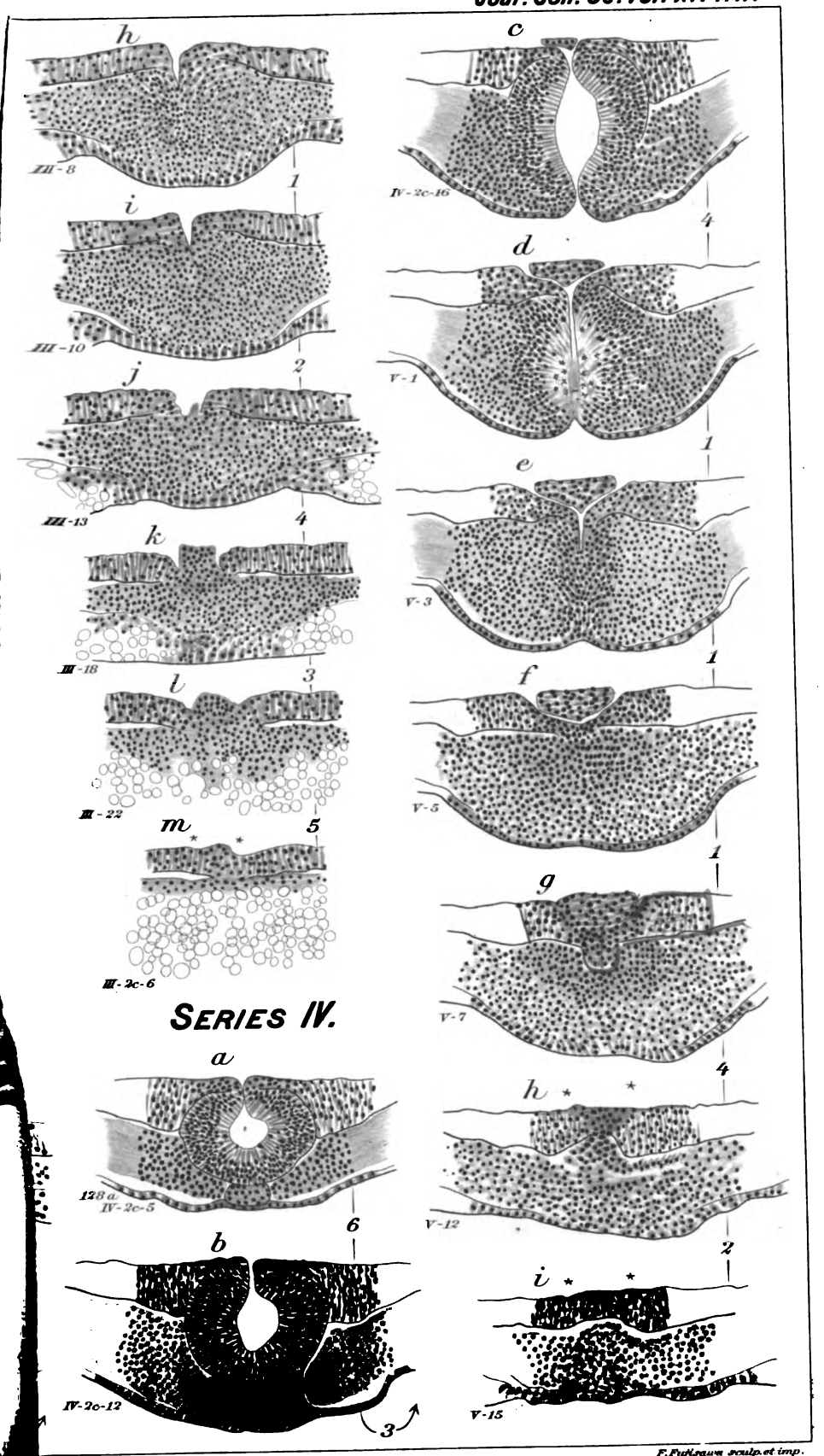

F. Fujisawa sculp. et imp.

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PLATE V.

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Plate V.

Arabic numerals placed between every two sections indicate the number of sections that intervene between those two (exclusive).
Certain marks on the left of each section are for the identification of the sections and do not concern the reader.

Series V. ***a–n.***—Sections through an embryo of *Chelonia caouana* with 9 mesoblastic somites, taken out 9½ days after deposition. Surface views given in Figs. 5 (Pl. I), 5*a* (Pl. II) and 5*b* (Pl. I). For detailed description see p. 44. *DD* × 2. (B 11 ハ)

Series VI. ***a–j.***—Sections through an embryo of *Trionyx japonicus* with about 10 mesoblastic somites. taken out 8½ days after deposition. Surface view given in Fig. 22, Pl. III—also similar to *Contrib. II*, Pl. III, Figs. 19 and 19*a*). For detailed description see p. 48. *CC* × 2. (143 田)

Series VI. ***long.***—Posterior portion of a longitudinal section through an embryo of *Trionyx japonicus* taken out from the same deposit at the same time as that of the above series. *DD* × 2. (143 *long.*)

The arrow indicates with its point the anterior end of the section. The lines marked *g*, *h*, *i* denote the planes through which the transverse sections marked by the same letters pass through.

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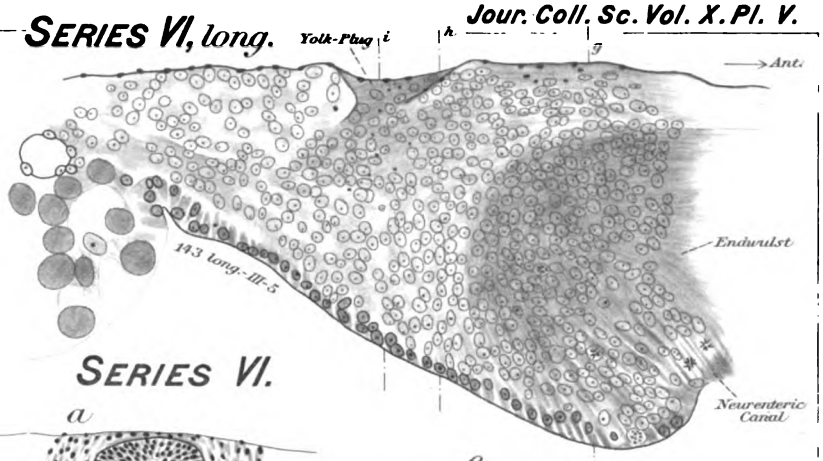
SERIES VI, long.



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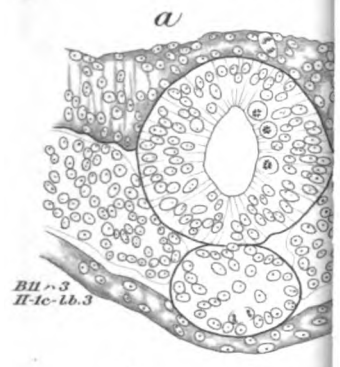

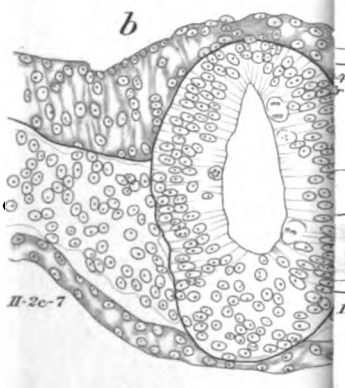

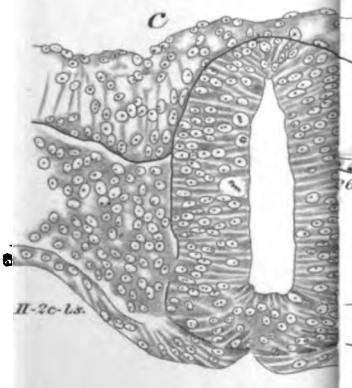

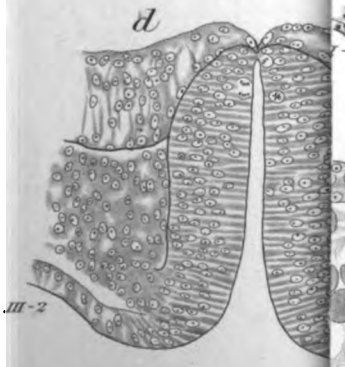

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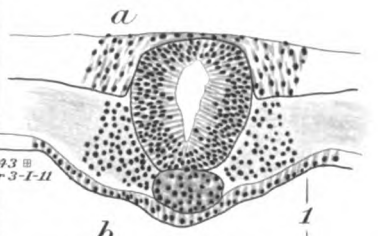

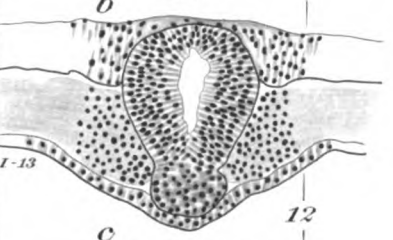

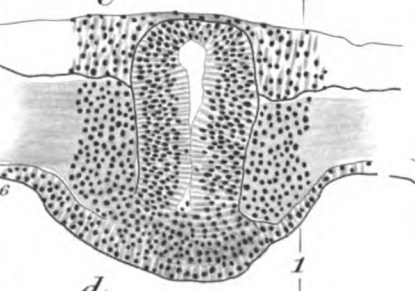

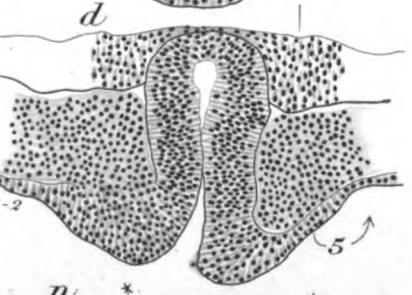

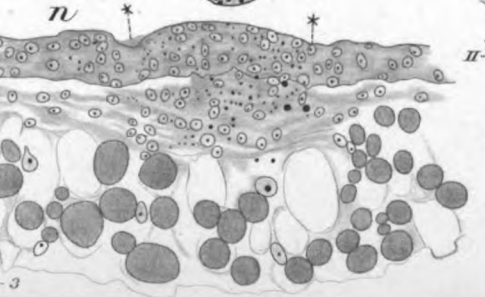

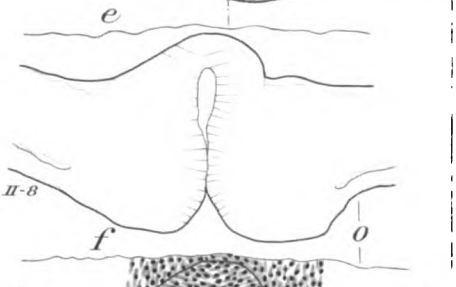

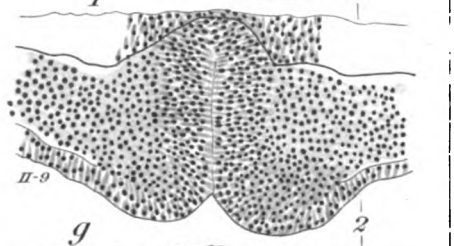

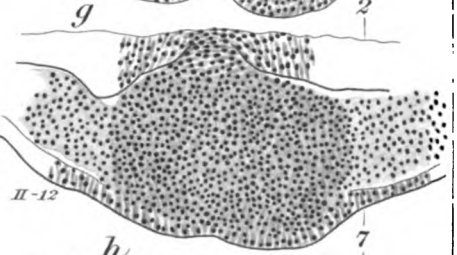

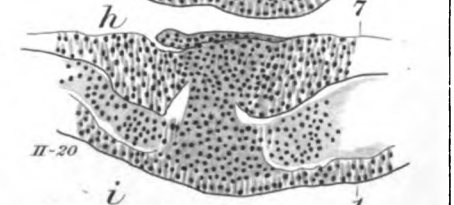

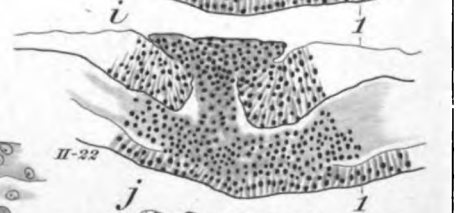

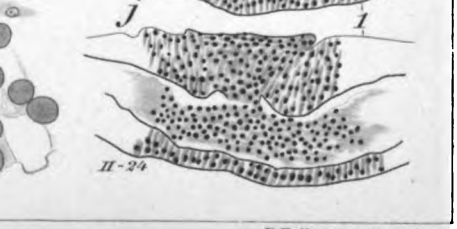

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PLATE VI.

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Plate VI.

Arabic numerals placed between every two sections indicate the number of sections that intervene between those two (exclusive). Certain marks on the left of each section are for the identification of the sections and do not concern the reader.

Series VII. *a*.—Section through an embryo of *Trionyx japonicus* taken out 7½ days after deposition. Surface view in Fig. 24 (Pl. II), in general appearance very much like Figs. 22 or 23. For detailed description see p. 50. *CC* × 2. (155 □)

Series VIII. *a–g*.—Sections through an embryo of *Trionyx japonicus* with about 13 mesoblastic somites, taken out 9½ days after deposition. Surface view given in Fig. 25 (Pl. III). For detailed description see p. 51. *CC* × 2. (151 ⊞)

Series XII. *a–j*.—Sections through an embryo of *Trionyx japonicus* with about 15 mesoblastic somites, taken out 10½ days after deposition. Surface views given in Figs. 26 (Pl. III), and 26*a* (Pl. II). For detailed description see p. 64. *CC* × 2. (157 ⊞)

Coel.'=Extra-embryonic cœlomic cavities.

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PLATE VII.

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Plate VII.

Arabic numerals placed between every two sections indicate the number of sections that intervene between those two (exclusive). Certain marks on the left of each section are for the identification of the sections and do not concern the reader.

Series IX. *a–n.*—Sections through an embryo of *Chelonia caouana* with about 16 mesoblastic somites, taken out 10½ days after deposition. Surface views given in Figs. 8 (Pl. I) and 8*a* (Pl. II). For detailed description see p. 53. *CC* × 2. (B 12 *1*)

Sedgwick ('92) Fig. 1—Embryo of *Scyllium canicula*, 2½ mm. in length. The hind end of the embryo is notched. The medullary groove is just beginning. The tail swellings of Balfour are well marked. (Sedgwick p. 585).

Sedgwick ('92) Fig. 2.—Embryo of *Raja?* sp., 4 mm. in length. The medullary groove is closed except at the hind end. The notched embryonic part of the edge of the blastoderm has grown faster than the rest, and come to project over the surface of the yolk. The sides of this projection are already slightly bent ventralwards. They will eventually meet and form the ventral part of the caudal region of the body (Sedgwick p. 585).

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PLATE VIII.

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Plate VIII.

Arabic numerals placed between every two sections indicate the number of sections that intervene between those two (exclusive).
Certain marks on the left of each section are for the identification of the sections and do not concern the reader.

Series X. *a–n.*—Sections through an embryo of *Clemmys japonica* with about 10 mesoblastic somites, taken out 6½ days after deposition. For detailed description, see p. 57. *CC* × 2. (LI ⊞)

(The numeral between *k* and *l* should be 4 instead of 5.)

Series X. *long.*—Posterior portion of a longitudinal section through an embryo of *Trionyx japonicus*, taken out from the same deposit at the same time as that of Series X. *CC* × 2. (LI *long*)

Epibl. = Epiblast. *Mesobl.* = Mesoblast.

Hypobl. = Hypoblast. *Medull. Canal.* = Medullary canal.

The lines *e–k* denote the planes through which the transverse sections marked by the same letters pass through.

Series XII. *long.*—Posterior portion of a longitudinal section through an embryo of *Trionyx japonicus*, taken out from the same deposit at the same time as that of Series XII, (Pl. VI). *CC* × 2. (157 *long*)

Amn=Amnion *b. v.* = Bloodvessels.

Coel.'=Extra-embryonic cœlomic cavity. *yk.* = Yolk.

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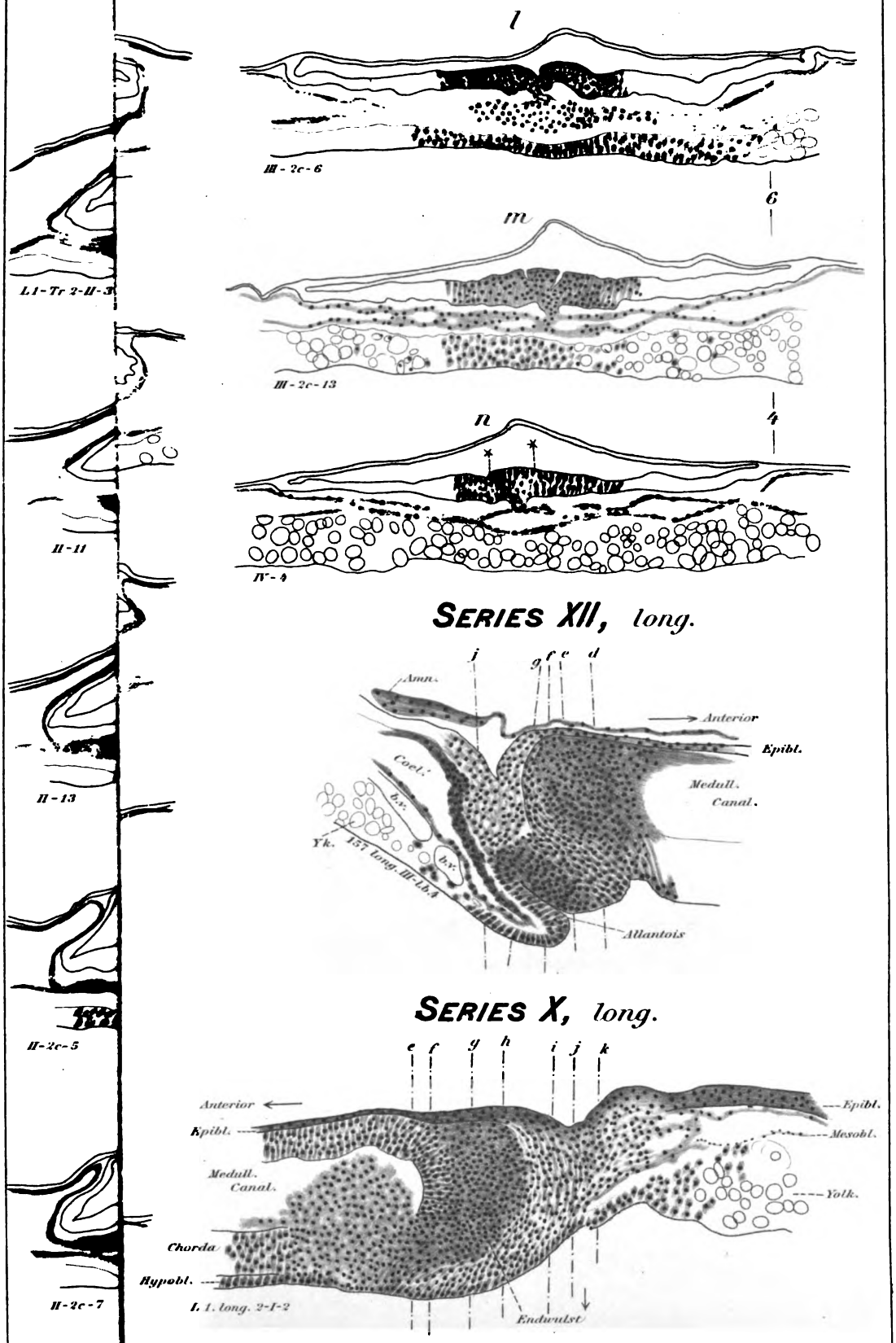

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Certain marks on the left of each section are for the identification of the sections and do not concern the reader.

Series XI. *a–o.*—Sections through an embryo of *Clemmys japonica* with about 18 mesoblastic somites, taken out 8 days after deposition. Surface view like Fig. 15 (Pl. II), For detailed description see p. 60. *CC* × 2.

(XXXXIII ⊕)

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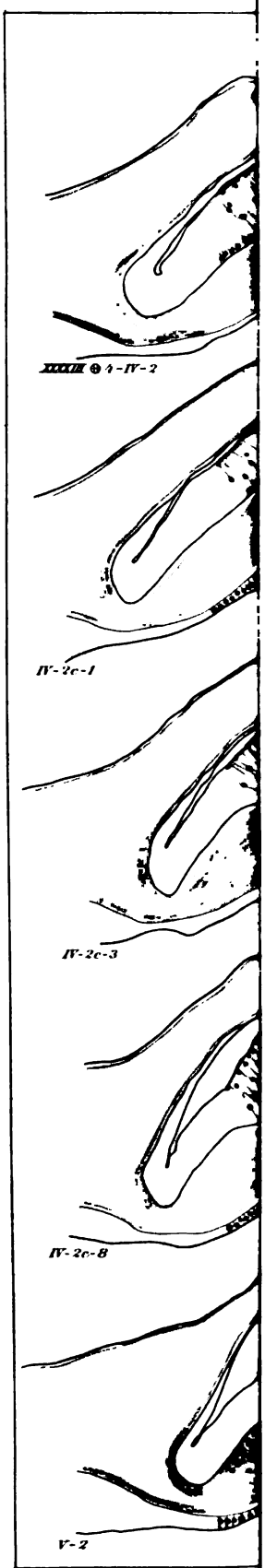

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PLATE X.

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Plate X.

Arabic numerals placed between every two sections indicate the number of sections that intervene between those two (exclusive). Certain marks on the left of each section are for the identification of the sections and do not concern the reader.

Series XIII. *a–l.*—Sections through an embryo of *Chelonia caouana*, with about 18–19 mesoblastic somites, taken out 11½ days after deposition; in general appearance very much like the *Clemmys* embryo given in Fig. 15. For detailed description see p. 65. *CC* × 2. (B 13 ¤)

Series XIII. *m.*—The fourth section behind *l* in the above series, shows the remnant of the yolk-plug. For detailed description see p. 67. *DD* × 2.

Amm. cav.=Amniotic cavity.

Coel'=Extra-embryonic body-cavity.

Coag. mass=Coagulated mass.

Epibl.=Epiblast.

Mesobl.=Mesoblast.

Sero-Amn. Conn.=Sero-amniotic connection.

Diagrams I–IX.—

Diag. I–IV show the course of development in Elasmobranchii.

Diag. V–IX show the course of development in Chelonia.

For detailed description see p. 101 *et seq.*

Ym. 1=Primary yolk-mass.

Ym. 2=Secondary yolk-mass.

Diag. IV*a* shows the tail part in IV on a larger scale.

Diag. IX*a* „ „ IX „ „ .

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SERIES XIII.

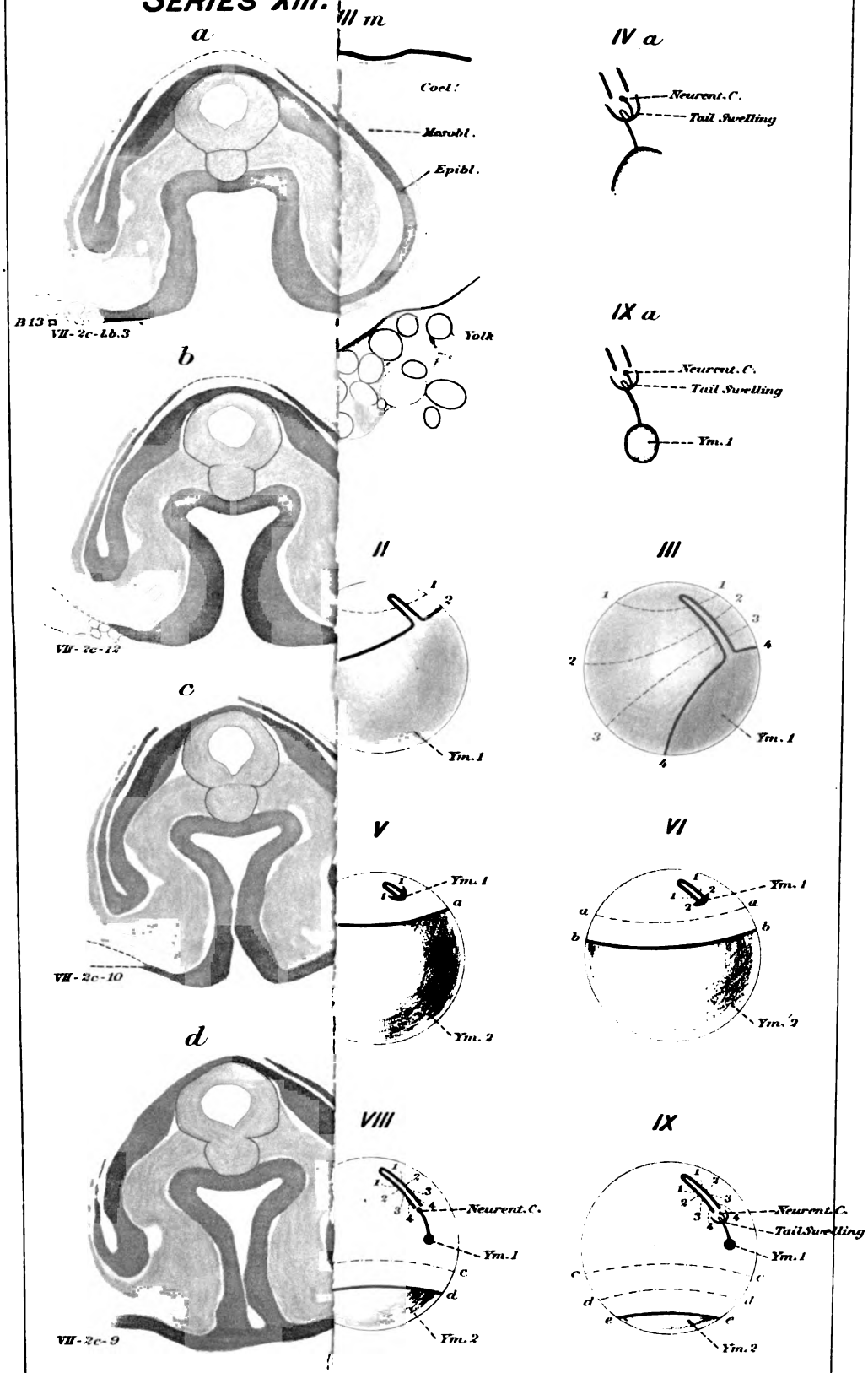

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PLATE XI.

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Plate XI.

Arabic numerals placed between every two sections indicate the number of sections that intervene between those two (exclusive). Certain marks on the left of each section are for the identification of the sections and do not concern the reader.

Series XVI. *a–z.*—Sections through an advanced embryo of *Chelonia caouana* taken out 16 days after deposition, with five gill slits all formed, the cranial flexure well marked, and the appendages knob-like. About the stage of *Contrib II*, Fig. 11 and 11*a*. For detailed description see. p. 68. *a–p* *aa* × 2; *q–z AA* × 2. (F7*a*)

Sero-Amn. Conn = Sero-amniotic connection.

Coel.' = Extra-embryonic body-cavity.

(The numeral between *j* and *k* should read 0 instead of 6.)

Series XIV. *k'*—The ventral median part of the tail in Section *k* in the above series more highly magnified. *DD* × 4.

Series XIV. *l'*—The ventral median part of the tail in Section *l* in the above series more highly magnified. *DD* × 4.

Hyp. = Hypoblast.

Mes. = Mesoblast.

Epi. = Epiblast.

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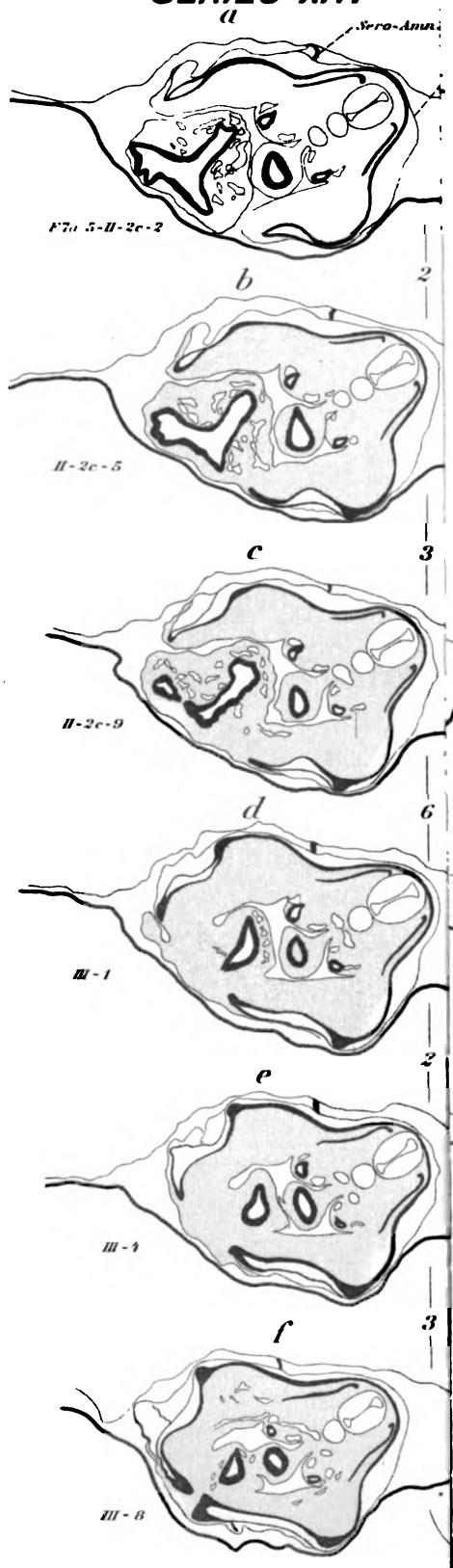
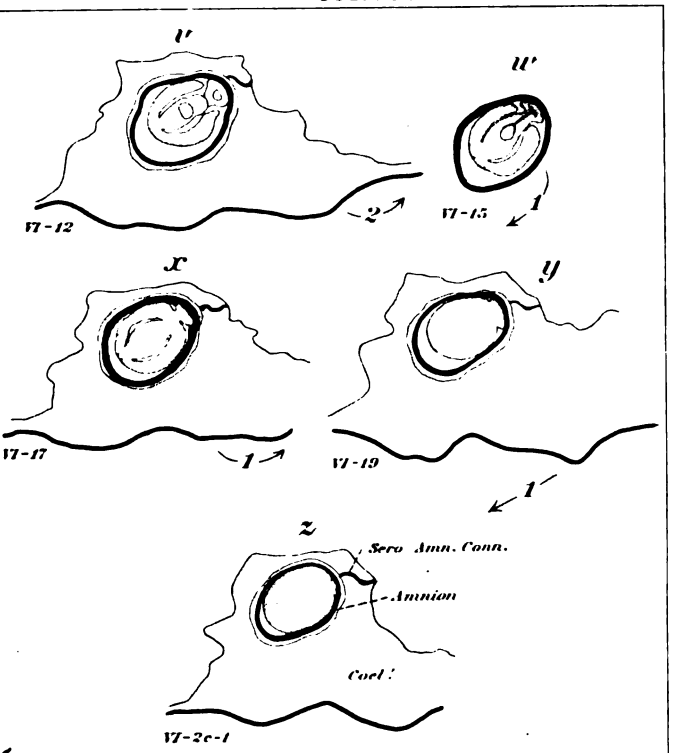
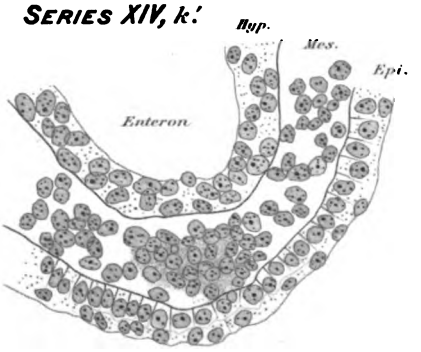
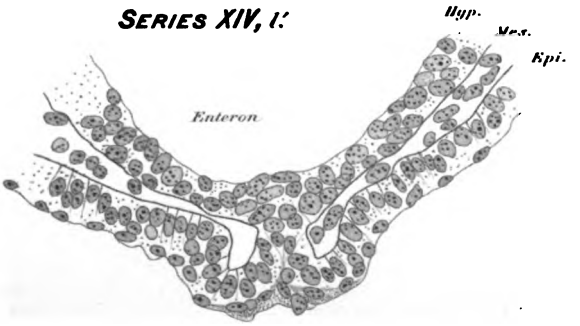
SERIES XIV.









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明治二十九年十月十九日印刷
明治二十九年十月廿二日發行

編纂兼發行者　帝國大學

印刷者　東京市日本橋區兜町貳番地
東京印刷株式會社
星野錫

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ANTHROPOLOGICAL SOCIETY LONDON

帝國大學紀要

理科

第十冊第二號

THE

JOURNAL

OF THE

COLLEGE OF SCIENCE,

IMPERIAL UNIVERSITY,

JAPAN.

VOL. X., PART II.

帝國大學印行

PUBLISHED BY THE UNIVERSITY.

TŌKYŌ, JAPAN.

1897.

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Ueber eine in Misaki vorkommende Art von Ephelota und über ihre Sporenbildung.

von

Dr. C. Ishikawa.

Professor der Zoölogie an der Kaiserlichen Universität zu Tōkyō.

Hierzu Tafeln XII und XIII.

Während eines kurzen Aufenthalts in der marinen Station des „Science College" in Misaki am Ende vorigen Jahres, hatte ich Gelegenheit, grössere Exemplare einer *Ephelota*art zu beobachten, welche in Milliarden auf den flottierenden Blättern, Stielchen, Luftbehältern u.s.w. einer *Sargassum*art vorkommt die in den Wintermonaten in einer kleinen Bucht auf der Ostseite der Meerestrasse zwischen unserer Station und der kleinen Insel Jogashima wächst. Diese ziemlich grossen Thierchen sind gelb-rötlich gefärbt und man kann sie leicht mit unbewaffnetten Augen von dem Kahne aus beobachten, wie sie auf den flottierenden Algeblättern sitzen. Sie sehen wie kleine Köpfchen von *Hydroidpolypen* aus, mit dem man leicht verwechseln kann, wie ich sie anfangs auch für solche gehalten habe.

A. Beschreibung des erwachsenen Thiers.

Die Gestalt des Körpers ist recht verschieden. In völlig ausgestrecktem Zustande gleicht das Thier, von den Seiten angesehen, einem etwas plattgedrückten Hexagon ; die Oberseite ist breiter wie die untere, wo es sich in ein langes Stielchen fortsetzt.

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1) Das Ectoplasma erscheint im lebenden Individuum als eine dünne, durchsichtige, körnchenfreie Zone. In gut conservierten Exemplaren kann man in dieser Zone drei Schichten unterscheiden ; a, eine äussere dünne, hyaline Schicht ; b, eine mittlere stark mit Eisenlackhæmatoxylin färbbare membranöse Schicht ; und c, eine innere auch dünne, hyaline Schicht, die ohne bestimmbare Grenzen in das Endoplasma übergeht. Die zwei äusseren dieser Schichten entsprechen der Pellicula der Autoren, während die innerste dem Corticalplasma. In der äussersten Schicht findet man eine grosse Zahl von stäbchenähnlichen Gebilden (Fig. 4b), die auf dem Durchschnitte des Körpers wie eine Reihe von Granula aussehen (Fig. 2a, b, und 4a). Diese Stäbchen sind aber nicht gleichmässig über die ganze Oberfläche des Körpers vertheilt, sondern alternierend in dichteren und dünneren Streifen, wie man in Fig. 4b deutlich sieht. Auch die einzelnen Stäbchen liegen nicht in beliebiger Richtung, sondern die meisten liegen in der Längsrichtung des Körpers. Von der Bedeutung dieser Gebilde kann ich ebensowenig sagen wie meine Vorgänger ; es scheint aber, dass sie nichts zu thun haben mit einer Alveolarschicht unter der Pellicula wie BÜTSCHLI (3) vermuthet, da man wie gesagt eine deutliche Membran zwischen den Stäbchen und darunterliegenden Corticalplasma zu unterscheiden pflegt (Fig. 4a).

2) Das Endoplasma enthält wie in allen verwandten Arten zahlreiche gefärbte und ungefärbte Körnchen von wechselnder Grösse und Gestalt. Die gefärbten Körnchen sind meistens von einem gelblichen oder röthlichen Braun, viele darunter aber mit bläulichem Ton. Behandelt wurde das Thierchen mit Eisenlackhæmatoxylin, viele der Körnchen zeichnen sich durch starke Färbbarkeit aus, so dass sie fast so aussehen wie die Chromatinblöckchen in dem Kern. Auch die Osmiumsäure verschwärzt einige dieser Körnchen wie man an den mit FLEMMING'S Flüssigkeit fixierten Exemplaren leicht erkennen

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kann. Das Endoplasma enthält auch viele Vacuolen von verschiedener Grösse. Diese stehen in der apicalen Fläche des Körpers so dicht nebeneinander, dass sie dem Protoplasma ein schaumiges Aussehen verleihen (Fig. 3). Sie sind aber nicht auf der ganzen Fläche zerstreut, sondern zu einem Kranz an den Saugtentakelnreihe angeordnet. Eine Reihe von grösseren Vacuolen findet man unter dem Corticalplasma zwischen der Basis der zweiten Tentakeln (t^2) am apicalen Zweidrittheil des Körpers (Fig. 2, 3, 4, 10, 13 und 15). Ueber die Contraktilität der Vacuolen habe ich keine sicheren Beobachtungen gemacht, obwohl einige dieser grösseren Vacuolen sehr langsam sich zu contrahieren scheinen.

3) Von den Tentakeln lassen sich fünf Gruppen unterscheiden. Eine Reihe von sehr langen Greiftentakeln (Fig. 1, t^1) steht am obersten Rand des Körpers, eine zweite am mitteren (Fig. 1, t^2), eine dritte Reihe von kleineren Tentakeln findet man am Basalende des Körpers um den Stiel. Innerhalb der ersten Tentakelnreihe findet man eine Gruppe von Saugtentakeln (t^4), auch zu einem Kranz angeordnet. Zwischen diesen anderen stehen ganz vereinzelt kleinere Tentakeln, die ich als Tentakeln V bezeichnen möchte (t^5). Die zwei grossen Greiftentakeln (t^1 und t^2) verhalten sich ähnlich in ihrer Bewegungsart ; sie lassen sich aber in morphologischer Hinsicht, in ihrer Grösse und besonders in dem Aufbau ihrer basalen Endes, leicht von einander zu unterscheiden. Was ihre Länge betrifft, so vermögen die apicalen Tentakeln (t^1) in ihren völlig ausgestreckten Zustande eindritt- bis einhalbmal die Länge zu erreichen wie die mittleren (t^2) im gleichen Zustande. Mit dem kürzeren Durchmesser des Körpers, sind die ersteren zwei-bis dreimal so lang. An der Basis unterscheiden sich beide Tentakeln von einander dadurch, dass die unteren (t^2), gleich wie die Tentakeln mehrerer anderen verwandten Arten, plötzlich aus der Oberfläche des Körpers entspringen, während die

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Tentakeln der oberen Reihe eine sehr verdickte Basis besitzen, etwa wie die Basalstücke der von CLAPARÈDE und LACHMANN (4, 6) beobachteten Tentakeln von *Tokophrya Trold*, wenn auch in unserer Art der feinere Endtheil allmählig zu dem verdickten Basaltheile übergeht. Die Zahl der beiden Tentakeln beziffert sich in grösseren Exemplaren auf dreizig bis vierzig. Die dritte Reihe der Tentakeln (t^3) entspringen, wie gesagt, um die Basalfläche des Körpers ganz um die Stielchen, wo der Körper stark eingewölbt ist (Fig. 2, 3). Diese Tentakeln sind kürzer und viel dünner wie die beiden anderen. Die Saugtentakeln die man innerhalb der Tentakeln I zu finden sind, sind in zwei bis drei Reihen angeordnet (Fig. 1, 2, 3 und 4 t^4). Dieselben sind kurz und dick wie in allen anderen Arten der Gattung, und jeder von dieser endet in einem deutlichen Knopf.

Der feinere Bau der Tentakeln weicht wesentlich von den bis jetzt beobachteten Fällen ab, oder vielmehr wir können jetzt, dank dem grossen Fortschritt der modernen Technik, den feineren Bau der Tentakeln viel genauer ermitteln wie es in früheren Zeiten der Fall war. Alle Tentakeln sind von der obenerwähnten äusseren Schicht des Ectoplasmas umgeben bis zur ihrem extremen Ende, nur sind sie nicht von den Stäbchen begleitet (Fig. 2a, b und 4a). Besonders deutlich treten die Verhältnisse in Fig. 2c hervor. Es verlohnt sich aber den Fall näher zu erklären, den ich in Fig. 2a dargestellt habe. Diese Figur macht auf den Beobachter den Eindruck als ob das ganze Ectoplasma von dem Tentakel durchbohrt ist, wie R. HERTWIG (5, 3) bei seiner *Ephelota gemmipara* vermuthet. Auch ich habe einen ähnlichen Eindruck bekommen, als ich zum ersten Mal das Präparat sah; genauere Beobachtungen zeigten mir aber, dass dieses nicht der Fall ist. Die Tentakelbasis ist stark eingestülpt, wie auch an dem Schnitt Fig. 5 zu sehen ist. Die Bedeckung mit der äussersten Schicht des Ectoplasmas auf der ganzen Länge des Tentakels ist vermutlich auch

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in der europäischen *Ephelota gemmipara* der Fall, und BÜTSCHLI (3) hat das Richtige getroffen, wenn er das Vorhandensein einer dünnen Pelliculaschicht, unter Aufgabe der Ansicht von der Stäbchenstructur, bis zu den Ende der Tentakeln auch in jener Art vermuthet.

In Innern der Tentakeln findet man Fadenstructuren die in den beiden grossen Greiftentakeln (t^1 und t^2) gleich stark entwickelt sind. Diese Fäden bestehen in den beiden Tentakeln aus vier parallel-laufenden Streifen, jede dieser wieder aus zwei feineren zusammengesetzt (Fig. 2 und 3, besonders deutlich in Fig. 2a). Die vier Fäden dieser Tentakeln sind nicht zu einem runden Bündel vereinigt, sondern sie liegen in einer Reihe senkrecht zur Horizontalebene des Körpers wie man aus den Figuren 2, 3, 4 und 5 deutlich sieht. Dementsprechend ist der Durchschnitt dieser Tentakeln nicht rund, sondern eine langgezogene Ellipse. Diese Fäden, wenn einmal erkannt, kann man auch in lebenden Exemplaren als hyaline Streifen im Inneren der Tentakeln beobachten. Besonders schön und deutlich treten sie, aber, in dem mit FLEMMING'scher Flüssigkeit conservierten und mit Eisenlackhæmatoxylin gefärbten Präparaten hervor, indem sie als sehr dunkel gefärbte Fäden erscheinen. In solchen Präparaten (Fig. 2, 3, 4 und 5) kann man die Fäden ins Innere des *Suctoria*körpers verfolgen. Die Fädenbündel der beiden Tentakeln stehen alternierend und kreuzen sich mit einander in der Ebene ein wenig unterhalb der Basis der Tentakeln II (vergleiche Figuren 2 und 3 mit dem Fig. 5), aber sie stehen nicht mit einander in Verbindung. Wie tief aber diese Fäden ins Innere des Körpers hineindringen und wie sie endigen, sind Punkte die ich mit Bestimmtheit nicht entscheiden konnte. Sicher ist es aber, dass diejenigen der Tentakeln I (t^1) in schräger Richtung bis zur Basis des Körpers laufen, um dort in knäuelförmigen Massen zu endigen (Fig. 3). Diejenigen der Tentakeln II (t^2) kann man in

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Querschnitten (Fig. 3) bis zur Mitte des Körpers, und zuweilen auch noch weiter, verfolgen. Ob diese aber mit einander zu einem Netze verbunden sind, bleibt dahingestellt. In einem Präparat glaubte ich eine solche Structur vor mir gehabt zu haben, die betreffende Schnitt war aber so dick, dass man leicht die übereinander liegenden Fäden mit einem Netze verwechseln kann. Auch die knäuelförmigen Enden der Fäden der zweiten Tentakeln erscheinen in vielen Fällen zu einem Netze verbunden zu sein.

Aehnliche Fadenstructuren findet man auch in den zwei anderen Tentakelarten—in den Basaltentakeln und in den vereinzelten Tentakeln zwischen den anderen. Diese sind aber von unbedeutender Grösse und auch in weit geringerer Zahl vorhanden, nur einer oder zwei in jeden Tentakel (Fig. 2 und 3 t^3 und t^4). Die gleichen Gebilde scheinen in den Saugtentakeln zu einem Röhrchen umgewandelt zu sein, wie man ganz deutlich in Fig. 2, 2c und 3 sieht. In Fig. 4 sieht man Querschnitte dieser Röhrchen wie sie im Innern des Körpers liegen (Fig. $4t^4$). In vielen Fällen kann man diese Röhrchen bis nahe zur Basis des Körpers verfolgen (Fig. 3). Ob aber alle diese Röhrchen so weit in den Körper eindringen, bleibt noch zu erforschen. Auch die Endigungsmodi dieser Röhrchen muss ich leider unbestimmt lassen.

Die Frage wie die Tentakeln aus dem Körper des Thieres hinaustreten, glaube ich in vielen Fällen beantworten zu können. An der Basis der Tentakeln findet man ein deutliches Loch in der Pelliculamembran, durch das die Tentakeln hinaustreten (Fig. 2b und 2c). Ich halte deswegen die Beobachtung HERTWIG's für das Richtige, wenn er die Pellicula seiner *Ephelota*art als durchbohrt darzustellen suchte (5, 3).

4) In der Ebene die durch die Basis der Tentakeln II geht, findet man im Innern des Körpers einen Kranz von faserigen

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Gebilden die zwischen den Tentakeln II verlaufen und so die Basaltheile derselben verbindet. Einen Schnitt durch diese Ebene habe ich in Fig. 5 darzustellen gesucht. Fig. 2 zeigt einen Längsschnitt nahe der einen Seite des Körpers. In diesem Schnitt sieht man ganz deutlich die durchschnittenen Stücke der Tentakelbasis II, und dazwischen einen breiten Band dieser Fasern, welcher links und rechts durchschnitten ist (Fig. 2f). In dem medianen Längsschnitt der Fig. 3 wurde dieser Faserkranz in zwei Querschnitten (f) angetroffen. Die physiologische Bedeutung dieser Fäserchen ist nicht schwer zu errathen. Sie dienen gewiss als Stütze für die Festhaltung der Tentakeln, gerade wie ein mit Schnüren verbundener Hacken.

5) Der Nucleus zeigt keine Besonderheiten der Art. Er ist wie bei *Ephelota gemmipara* ein ringförmiger Körper von beträchtlicher Grösse mit vielen oberen und unteren Auswachsen, die manchmal sich noch weiter verzweigen. Auch kurze horizontale Zweige, wenn auch in geringer Zahl, werden von dem ringförmigen Mittelstück ausgesendet. Das ringförmige mittelere Stück des Nucleus liegt horizontal ungefähr in der Mitte des Körpers unter den Wurzeln der Tentakeln II, und umschliessen diejenigen der Tentakeln I. Die oberen Zweige steigen schräg nach oben um die Aussenseite des ringförmigen Faserbandes zwischen den Wurzeln der Tentakeln II, und so in der Ebene mit den Wurzeln der Tentakeln I (Fig. 3 und 5). In dem lebenden Thierchen kann man den Kern nicht leicht beobachten, da der Körper ganz mit gefärbten Körnchen gefüllt ist. Färbt man das Thierchen aber mit Reagentien, so tritt die Grundsubstanz des Kernes als homogene Masse hervor, worin man zahlreiche Chromatinblöckchen erkennen kann. Diese letztere sind von verschiedener Grösse und Gestalt. Meistens sind sie oval oder länglichoval, zuweilen aber auch wurstförmig, stark gekrümmt oder auch eckig. Behandelt

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man den Schnitt mit Eisenlackhæmatoxylin, so färben sich die Chromatinblöckchen ganz schwarz, während die Grundsubstanz graulich erscheint. Mit Safranin-Hæmatoxylin oder mit Safranin-Orange nimmt das Chromatin die Safraninfarbe an, während die Grundsubstanz sich mit Hæmatoxylin oder mit Orange tingirt. Keine Streifungen der Grundsubstanz wurden in den ruhenden Kerne beobachtet.

Ausser diesem Kern, den wir mit BÜTSCHLI (3) als das Aequivalent des Grosskerns der *Ciliaten* ansehen können, finden wir in vielen Exemplaren kleine rundliche Gebilde nahe dem Mittelstück des Kernes liegend, die gerade so aussehen wie einzelne Chromatinblöckchen (Solche findet man unter den etwas schief liegenden Kernstück rechts von der Mittellinie im Schnitt Fig. 3). In dem mit FLEMMING'scher Flüssigkeit fixierten Präparat lassen diese Gebilde sich schwer von den sonstigen Körnchen des Endoplasmas unterscheiden, da einige von den letzteren wie gesagt sehr stark mit Osmiumsäure geschwärzt wurden, so dass sie sehr leicht verwechselt werden können. Immerhin aber sind sie durch den Farbenton von den Fettkörperchen zu unterscheiden, wenn man sie genau betrachtet. In Sublimat-Essigsäure Präparaten wo die meisten Endoplasmagranula gewöhnlich verschwinden, findet man die fraglichen Gebilde ganz deutlich hervortretend. Diese lassen sich aber wie gesagt nicht in allen Präparaten nachzuweisen. Demnach kann man nicht mit aller Sicherheit schliessen, dass sie den Micronuclei der *Ciliaten* entsprechen, obwohl es sehr wahrscheinlich ist. Alle Bemühungen die Theilungsphænomene dieser Gebilde zu beobachten, wodurch man die sichere Beweise ihrer micronuclearen Natur bekommen könnte, waren vergebens. Nur vereinzelt traf ich diese Gebilde in den sich in Knospung begriffenen Exemplaren, aber alle diese waren auch im ruhenden (?) Zustande vorhanden (Fig, 11n?).

6) Der Stiel erreicht in vielen Exemplaren zwei bis dreimal die

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Körperlänge und ist am apicalen Ende, wo er mit dem Körper zusammenhängt, stark verdickt (Fig. 1, 3 und 4). Er verjüngt sich allmählig nach seinem distalen Ende, wo er in einer, mit einem verdickten Rand versehenen, membranösen Scheibe endigt. Diese Scheiben lassen sich intensiv mit Carmin färben, wie HERTWIG (5, 3) schon beobachtete. Eine Anzahl von stäbchenähnlichen Structuren wurde auch an dem Scheibenrand beobachtet, gerade so wie bei HERTWIG'schen Exemplaren. Jedoch halte ich die Scheibe nicht für ein Product des veränderten Perisarc des *Hydroid*enkörpers wie HERTWIG, sondern ich glaube, dass sie ein Äquivalent ist für die basale Befestigungsscheibe der *Vorticelliniden*, da bei unserer Art, die nicht auf einem *Hydroid*körper sitzt wie seinige, die Scheibe sich wie gesagt gut mit Carmin färben lässt. Beweiskräftig für diese Ansicht ist aber das Vorhandensein einer sehr gut entwickelten Scheibe mit stark verdicktem Rand am basalen Ende des frei im Wasser hervorragenden Stiels in jungen Thierchen wie wir in Figuren 14 und 19 sehen; desshalb schliesse ich mich ganz MAUPAS an (7), wenn er den Ring als die in *Suctorien* vermissten Basalscheibe der *Vorticelliniden* betrachtet.

Von den feineren Structuren des Stieles vermag ich nichts neues anzugeben. Er besteht aus einer hyalinen structurlosen Hautschicht und einer relativ dicken Markschicht, welche letztere feine Längs-und ferner nicht sehr deutliche Querstreifungen zeigt. Mitten durch die Markschicht bemerkt man in jungen Thierchen einen geräumigen Kanal, der aber in vielen erwachsenen Thieren ganz zu verschwinden scheint (vergleiche Fig. 16 mit den Längsschnitten Fig. 3).

7) Der grösste Längendurchmesser eines wohl entwickelten Thieres mass 0.20 mm; der grösste Querdurchmesser 0.25 mm.

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B. Die Sporenbildung.

1) Die bewimperten Sprösslinge entstehen in Allgemeinen ebenso wie bei *Ephelota gemmipara*. Die erste Veränderung geht wie dort in dem apicalen Ende der aufsteigenden Zweige des Kernes vor sich. Wenn die Sprösslinge wachsen, so nehmen sie gewöhnlich eine elliptische Gestalt an mit einer stark ausgehölten Seite. An dieser Seite, die der Medianachse des Mutterkörpers zugekehrt ist, findet man eine reichliche Bewimperung (Fig. 7, 8, 9, 10 und 12), wie bei jener. Diese Sprösslinge weichen aber von denen der europäischen darin ab, dass bei unserer, die Greif-sowie Saugtentakeln schon auf ihrer Aussenseite beobachtet werden, wenn sie noch nicht von dem Mutterkörper losgelöst sind (Fig. 10), während die frei-schwimmenden Sprösslinge von *Ephelota gemmipara* tentakellos sind. In seiner sonstigen Gestalt stimmt der einzelne Sprössling fast mit der von *Ephelota gemmipara* überein; vielleicht ist es ein wenig dicker. Auch ist die ventral Vertiefung ungefähr dieselbe wie bei jener Art (Fig. 7, 8 und 9), nur die tiefsten Stelle, wo die Stielanlage sich entwickelt, liegt nicht am vorderne Ende des Körpers, sondern etwa in der Mitte (Fig. 12). Diese Stielanlage stellt sich zuerst als eine rundliche Vertiefung an der betreffenden Stelle des Körpers dar, und zeichnet sich durch starke Färbbarkeit des umgebenden Körperplasmas aus, gerade sowie die basale Scheibe des fertigen Stiels, ein Umstand der ebenfalls für die oben besprochene Natur des Stielendes spricht. In Sprösslingen, die eben vom Mutterkörper losgelöst sind, und die im Wasser herumschwimmen, kann man die Stielanlage nicht beobachten. Conserviert man aber solche Sprösslinge mit irgendwelchen Reagentien, so tritt die Stielanlage durch ihre intensive Farbaufnahme deutlich hervor. Ein in Fig. 6 dargelegter Sprössling representiert

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eine von den drei Sporen, nach Behandlung mit SCHNEIDER's Essigsäurecarmin. Dasselbe wurde bei gleicher Vergrösserung abgezeichnet wie die drei lebendigen (Fig. 7, 8 und 9), ist aber stark durch die Essigsäure gequollen und auch durch dem Deckglass abgeflacht. Die Greif-sowie die Saugtentakeln, den Kern und die zwei grossen Vacuolen sieht man sehr deutlich, besonders aber das kurze Stielchen in der ventralen Vertiefung und an dessen freien Ende eine mit Carmin stark gefärbten Scheibe, an deren verdicktem Rand ein ziemlich langer Wimperkranz (w k) hervortritt. An in FLEMMING's Flüssigkeit conservierten und mit Pikrocarmin gefärbten Präparaten kann man bei geeigneter Tubuseinstellung diese sämmtlichen Gebilde ebenfalls beobachten, natürlich aber nicht so deutlich und auch nicht alle in einem Ebene wie bei jenem.

Die grösste Zahl der Sprösslinge habe ich auf zwölf geschätzt, die kleinste auf vier.

2) Die Stiel-tragende Spore.

Die Sprösslinge, die wir soeben geschildert haben, schwimmen mittelst ihrer Cilien im Wasser umher, sobald sie von den Mutterkörper losgelöst sind. Es findet sich aber bei unseren Thierchen noch eine zweite Art von Sprösslinge, welche die ganze Gestalt des erwachsenen Individuums annehmen, wenn sie noch mit dem Mutterkörper zusammenhängen. In ihrer ersten Anlage zeichnen sich diese Sporen durch keine Besonderheiten von den anderen aus. In gleich grossen Sprösslingen beiden Arten findet man aber einen erheblichen Unterschied in ihren Tentakeln, Cilien und insbesondere in den Stielchen wie man auf Fig. 10 und 19 ganz deutlich beobachten kann. Wie man aus den Figuren 12 und 16 ersieht, sind alle Tentakeln schon wohl entwickelt, und auch diese haben die nämliche Stellung wie beim erwachsenen Thiere. Nur die Tentakeln III vermissen wir in diesen beiden Figuren. Das sie aber

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ebenfalls vorhanden sind, und nur dem Gesichtfeld verborgen bleiben, zeigen die Schnitte, die wir in den Figuren 13, 15, 17 und 18 repräsentiert haben. Dagegen die Cilien, die wir so entwickelt in anderen Sporen am ihrer ventralen Fläche beobachten können, sind in unseren Exemplaren bei gleicher Vergrösserung kaum zu sehen. Bei ZEISS' Objectiv D mit dem Ocular 2, wobei wir in den anderen Sporen die Cilien ganz deutlich beobachten können, sind sie in diesen Sprösslingen nicht oder nur sehr schwer wahrzunehmen. Schon aber mit dem Ocular 4 kann man die Bewimperung gut erkennen, wie wir sie auf dem Fig. 16 dargestellt haben. Das sie aber beträchtlich kleiner sind, zeigen auch die Figuren 10, 16 und 17. Von besonderem Interesse ist aber das Vorhandensein eines wohlentwickelten Stieles in diesem Sprössling wie man ihn in den Figuren 14, 14a, 15, 16, 17 und 19 dargestellt findet. Die Anfangsstadien dieser Stielchen habe ich leider nicht beobachtet; dass sie aber viel früher entwickelt sind wie bei den anderen Sprösslingen, zeigen auch die Figuren 10 und 19, wo bei den Sprösslingen, die wir in Fig. 10 dargelegt haben, nur eine ganz kleine Stielanlage entwickelt ist, während in den beiden Sprösslingen der Fig. 19 sie beinahe zweimal die Körperlänge erreicht. Der Wimperkranz auf dem freien Stielende dieser Sprösslinge ist aber ebensogut entwickelt, wie bei ungestielten Sporen. In lebenden Thierchen kann man die schwache Bewegung dieser Wimpern beobachten. Sie scheinen sehr klebrig zu sein, da wir sehr oft beobachtet haben, wie allerlei Schmutz daran angeklebt ist (Fig. 17). Ob sie aber zu stäbchenähnlichen Gebilden auf dem Stielende des erwachsenen Thieres direkt umgewandelt werden, muss eine offene Frage bleiben, da ich keine Beobachtungen über ihre Umwandlung machen konnte.

Die Stielchen wachsen senkrecht zu der dorso-ventralen Achse des Mutterkörpers wie man aus der Stellung der Sprösslinge wohl erschliessen kann, sie bleiben aber in dieser Stellung nur so lang, als

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sie in dem centralen freien Raum Platz finden. Werden sie aber länger, so können sie in dieser Richtung nicht mehr wachsen. In Folge dessen biegen sie nach oben um, wie man in den Figuren 14, 15, 16 und 18 ganz schön beobachten kann. Eine sehr interessante Thatsache in dieser Beziehung ist die, dass auch in solchen Fällen, wo die Zahl der Sprösslinge sehr klein ist, und ein weiter Raum für die gerade Ausstreckung des Stieles frei bleibt, die Stielchen doch in der nämlichen Richtung umbiegen, wie man aus der Fig. 19 erkennt. Angesichts solcher Thatsachen müssen wir annehmen, dass das Umbiegen des Stieles zuerst durch solche Ursachen veranlasst war, wie ich oben angegeben habe, dass aber in langen Generationen dieser Umstand in der Species so fixiert wurde, dass die Stielchen sich auch umbiegen, wenn sie keine Hindernisse für ihre weitere Entwicklung vorfinden.

Die Zahl dieser Sporen ist ebenso Aenderungen unterworfen wie die der anderen, je nach der Grösse des Mutterthiers. Die grösste Zahl, welche ich beobachtet habe, beträgt sechszehn (Fig. 14 und 14a), die kleinste nur eine. Zwischen diesen beiden Extremen kommen alle möglichen Uebergänge vor. Ueber die Lostrennung dieser Sprösslinge konnte ich leider nichts beobachten. Die Maximalgrösse des Sprösslinge, ehe sie sich vom Mutterkörper loslöst, kann ich daher auch nicht angeben. Ich glaube aber, dass sie nicht beträchtlich, von derjenigen die wir in Fig. 19 angegeben haben, abweicht.

3) Die Bildung der stieltragenden Sprösslinge, die wir soeben geschildert haben, ist von besonderem Interesse, da keine analogen Fälle bis jetzt in der ganzen Unterklasse der *Suctoria* beobachtet wurden. Ein einziger ähnlicher Fall, der mir bekannt ist, rührt von C. Robin (8, 3) her, der, nach der werthvollen Schilderung O. Bütschli's in seinem grossen *Protozoen* Werke, „bei *Ephelota gemmipara* gelegentlich apicale Knospen (1–4) beobachtet haben will,

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welche keine Cilien entwickelten und auch nicht die charakteristische Gestalt der Schwärmer annahmen, sondern niedere, cylindrische Form und kurze bis längere Tentakel besassen. Allmählich sollen sich diese Knospen verlängern und vom Mutterkörper mehr abschnüren; schliesslich entwickelten sie an ihrem Basalende einen kurzen, homogenen und farblosen Stiel, mit dem sie der *Ephelota* aufsassen." BÜTSCHLI zweifelt dabei ganz mit Recht, ob diese Gebilde, die ROBIN für Knospen zu halten glaubt, wirklich als solche betrachtet werden können, insoweit wie ihre allmähliche Entstehung aus dem *Ephelota*-körper noch nicht genauer erfolgt wurde „und namentlich auch die Beziehung zwischen ihren Kernen und dem der *Ephelota* noch nicht aufgeklärt ist." Obwohl der erste dieser Punkte in meinen Beobachtungen noch nicht genügend aufgeklärt ist, glaube ich doch, dass die Beziehungen des Kerns zwischen dem Mutterkörper und dem der Sprösslinge in unserem Fall ausser aller Zweifel klargelegt wurden (Fig. 13, 14a, 15 und 18), und das lässt vermuthen, dass was ROBIN gesehen hat, wirklich die Sporen waren; ob aber diese echte Stielchen getragen haben wie die der meinigen, darf wohl bezweifelt werden. Wie oben angegeben, entwickelt sich das Stielchen an der ventralen Aushölung des Sprösslings, und wächst zuerst senkrecht zu der dorsoventralen Achse des Mutterkörpers. Es krümmt sich aber gleich nach oben um, und endet frei im Wasser. Diese Lage des Stielchens an der ventralen Aushölung des Sprösslings findet sich nicht nur in unserer *Ephelota*art, sondern wie gesagt auch in der HERTWIG'schen Species. Aehnlich verhält es sich bei dem Sprössling der *Tokophrya quadripartita*. Nach den schönen Untersuchungen von BÜTSCHLI (2) wächst der äquatoriale Wimper-Ring dieses Sprösslings rings um die dorsoventrale Ebene des Sprosskörpers, während derselbe noch im Mutterkörper bleibt. Wenn der Sprössling aber losgelöst ist, so schwimmt er mit seiner früheren lateralen Seite nach unten gerichtet, um auf

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irgendwelcher Unterlage sich festzusetzen. Die abweichenden Angaben ROBIN's von der Basal-Lage des Stieles in seinem Thierchen scheint deswegen ganz unwahrscheinlich, besonders wenn der Sprössling mit diesem Stielchen auf dem Mutterkörper sitzen soll. Mir scheint es vielmehr, dass was ROBIN für das Stielchen des Sprösslings gehalten hat, in der That nicht ein solcher war, sondern eine protoplasmatische Brücke zwischen dem Sprosskörper und dem Mutterthier ehe der erstere sich losgelöst hat, wie man eine solche am basalen Ende des in Fig. 16 dargelegten, gestielten Spröslings ganz deutlich sieht. Manchmal beobachtet man eine solche Brücke in Gestalt eines langgezogenen Tentakels auch am basalen Ende der bewimperten Sprösslinge, wenn dieselbe im Begriffe sind, von dem *Ephelota*körper fortzuschwimmen (Fig. 9). Trifft diese Erklärung bei den ROBIN'schen Sprösslingen zu, so bleibt nur übrig, anzunehmen, dass diese nicht anders als unsere bewimperten Sprösslinge sind. Von Interesse ist aber die Thatsache, dass was ROBIN gelegentlich in der europäischen Art zur Beobachtung bekommen hat, in unseren Thierchen die normalen Fortpflanzungsmodi zu sein scheinen, wenigstens in der Colonie, die mir im letzten Winter in Misaki zur Untersuchung diente, während die tentakellosen, aber mit Wimpern versehenen Sporen jener Art, hier bei unseren Thieren ganz fehlen. Anstatt dessen giebt es bei unserer Art noch weiter entwickelte Sprösslinge, die mit einem echten Stiel versehen sind, was bei jener Art noch nicht zur Beobachtung kam. Die relative Anzahl der gestielten und ungestielten Sprösslinge habe ich nicht genau festgestellt, ich glaube aber ganz sicher, dass nicht weniger als ein-drittel von den sporentragenden Individuen mit gestielten Knospen versehen sind. Im Ganzen und Grossen daher kann man annehmen, dass die Fortpflanzungsmodi in unserem Thierchen einen höheren Entwicklungszustand darstellen, wie die der europäischen Art. Unerklärlich

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scheint mir aber die Wimperlosigkeit der ROBIN'schen Sprösslinge. Die Cilien entwickeln sich in allen bekannten Sporen der *Suctoria* eher als die Tentakeln. Die im Wasser frei schwimmenden HERTWIG'schen Sporen sind mit Cilien versehen, während unsere ungestielten Sprösslinge, wie oben angegeben, Cilien und Tentakeln tragen, aber nur die erstere dienen beim Schwimmen. Die Entwicklungszustände dieser Sporen zeigen uns, dass die Cilien sich viel früher entwickeln, als die Tentakeln, und auch in unseren gestielten Sporen finden wir eine wohlentwickelte Bewimperung. Diese Cilien sind aber, wie wir schon sahen, viel weniger entwickelt wie bei den stiellosen Knospen, und können aller Wahrschenlichkeit nach nicht für die Fortbewegung des Sprösslings dienen. Sie müssen daher als eine Reminiscenz der einmal so wohl entwickelten Cilien der anderen Sporen betrachtet werden, die aber durch die Entwicklung des Stieles allmählig im Verschwinden begriffen sind. Sollten vielleicht die ROBIN'schen Sprösslinge als ein noch weiter entwickelter Zustand, gegenüber den unseren gestielten Sprösslingen betrachtet werden? Darüber müssen die weiteren Untersuchungen jener Formen entscheiden.

Was schliesslich das Vorkommen der beiden Arten der Sprösslinge, die stiellosen und die gestielten, in einer Kolonie nebeneinander betrifft, so dürfte das auch nicht ohne Interesse sein. Ob irgendwie gewisse Umstände die Produktion der einen oder der anderen Form begünstigen, konnte ich bis jetzt nicht entscheiden. Auf einem und demselben Stielchen, Blatt oder Luftbehälter der *Alge* begegnet man den beiden Sporenarten in buntester Weise gemischt, so dass ihre Entwicklung kaum von äusseren Umständen abhängen kann. Natürlich wird es von grossen Nutzen sein für die Erhaltung der Art, wenn sie statt eine Sporenart zwei und zwar von ganz anders gebauten produciert, die daher auch verschiedenen physiologischen Zuständen unterworfen sind.

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4) Die Kernveränderungen während der Sporenbildung wurden nur in sehr wenigen Fällen beobachtet, obwohl ich viele Schnitte von den sporentragenden Individuen anfertigte. Was ich in der Fig. 11 dargestellt habe, ist das jüngste Stadium, das zu meiner Untersuchung gekommen ist, und kann ich leider die interessante Frage, was den ersten Anstoss zur Sporenbildung giebt, noch nicht lösen. Was ich aus diesen und anderen Schnitten schliessen kann, ist die Thatsache, das der Kern sich unter einem Knäuelstadium theilt, wobei das Kernplasma eine deutliche Faserung zeigt, wie man sie in der Theilung der Grosskerne der *Infusorien* gewöhnlich sieht. Die Apicalenden des Mutterkernzweiges biegen sich dabei gewöhnlich nach Aussen um, d.h. nach dem künftigen Apicalende des Sprosses, und bleiben in diesem Zustande bis sie wieder zum Apicalende des Tochterkerns werden. Diese Thatsache dürfte für die Orientiernng des Sprosskerns von Interesse sein, da der Sprosskörper wie gesagt ganz anders orientiert ist wie das Mutterthier.

Was für eine Veränderung die oben erwähnten kleinkernähnlichen Gebilde während dieser Zeit erleiden, kann ich leider wie erwähnt nicht angeben.

C. Systematische Stellung des Thierchen.

Die geschilderten Punkte lassen unser Thierchen von den bis jetzt bei *Ephelota*arten bekannten wohl mit Sicherheit unterscheiden. Am nächsten stimmt die Art aber mit der Hertwig'schen *Ephelota gemmipara* überein (5, 3). Sie unterscheidet sich aber von dieser Art durch das Vorhandensein von fünf verschieden gestalteten Tentakeln und durch deren regelmässige Lage am Thierkörper, sowie durch den Bau der Sporen. Bei der geringen Literatur, die mir hier zur Verfügung stand, kann ich natürlich nicht auf die Artbestimming eingehen. Sollte sie sich aber als eine neue Art erweisen, so möchte

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ich dem in höchsten Grade verdienstvollen *Protozoen*forscher Herrn Geheimen Hofrath Professor Dr. O. BÜTSCHLI dediciren, und sie *Ephelota Bütschliana* nennen.

Ende August, 1896.

College of Agriculture,
Imperial University.

Nachtrag. Während die vorstehende Abhandlung sich im Druck befand, erhielt ich durch die Güte des Herrn Dr. R. S. BERGH (1) in Kopenhagen eine sehr interessante Mittheilung über das Vorkommen von faserigen Gebilden in der Zellsubstanz zweier *Infusorien*arten, *Spathidium spatula* und *Holophrya Emmae*, die mit meinigen obenerwähnten Tentakelfasern Aehnlichkeit haben. Diese Fäserchen kommen in den beiden Arten in verschiedenen, aber ganz bestimmten Körperregionen vor. Vom Ectoplasma entspringend verlaufen sie in's Innere des Körpers, um im Endoplasma zu endigen. Auch die stäbchenähnlichen Structuren um die Schlundgegend vieler *Infusorien* wurden von dem Autor als homologe Structuren gedeutet. In wie weit aber diese Structuren mit den unserigen in Zusammenhang stehen, müssen künftige Forschungen lehren. Sollten vielleicht die Achsenfäden mancher *Heliozoën*, insbesondere diejenigen der von SCHAUDINN (9) neuerdings genau untersuchten *Camptonema nutans* ähnliche Gebilde vorstellen?

Literaturverzeichniss.

Diejenigen Schriften, die ich nicht im Original einsehen konnte, wurden mit einem* versehen.

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9. Schaudinn, F. :—Camptonema nutans nov. gen. nov. spec., ein neuer mariner Rhizopode. Separat Abdr. a. d. Sitzungsberichten d. k. preuss. Akad. d. Wissensch. zu Berlin. LII. 1894.

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Tafel XII.

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Tafel XII.

Ephelota spec. (?).

Sämmtliche Bilder sind mit einer Zeichencamera auf der Höhe des Objecttisches gezeichnet, und mit den Symbolen der dabei benutzten Oculare und Objective im Klammern beigefügt, wie $\left(\frac{2}{D} \times Z\right)$, $\left(\frac{4}{1/12} \times Z\right)$ u. s. w. die als Zeiss' Objectiv D mit Ocular 2, Zeiss' Objectiv 1/12 Immersion mit Ocular 4 u. s. w. zu lesen ist. *cl*, cilien; *f*, Fasergebilde im Endoplasma; *N*, Macronucleus; *Stb*, stäbchenähnliche Gebilde in der äussersten Schicht des Ectoplasmas; t^1, t^2, t^3, t^4 und t^5, Tentakeln.

Fig. 1. Mittelgrosses Individuum mit ausgestreckten Tentakeln, lebendig gezeichnet.

Fig. 2 und 3. Zwei Bilder aus einer Reihe von Längsschnitten von einem Individuum: Fig. 2 eine seitliche, und Fig. 3 eine mediane. Flemming's Flüssigkeit mit Eisenlack-Hæmatoxylin.

Fig. 2a. Kleines Stück des Tentakel I von einem anderen Exemplar, ebenso behandelt wie die vorigen.

Fig. 2b. Ebenso ein solches Stück von demselben Schnitt. Man beachte eine rundliche Oeffnung in der Pellicular Membran für den Austritt des Tentakels.

Fig. 2c. Drei Stückchen vom Saugtentakel von demselben Schnitt. Die äusserste Schicht des Ectoplasmas ganz deutlich bis zur Tentakelspitze zu beobachten.

Fig. 4 und 5. Zwei Durchschnitte von einem anderen Individuum, ebenso behandelt wie die vorigen: Fig. 4 in einer Ebene oberhalb der Tentakel II, und Fig. 5 unterhalb derselben.

Fig. 4a und 4b. Kleine Theile des Schnittes Fig. 4 noch stärker vergrössert: in Fig. 4a sieht man eine Durchschnittsansicht der Stäbchengebilde und in Fig. 4b eine Flächenansicht derselben.

Fig. 6. Bewimperter Sprössling soeben vom Mutterkörper befreit. Derselbe ist mit Schneider's Essigsäurecarmin stark gequollen.

Fig. 7, 8 und 9. Drei ebenso vom Mutterkörper befreite Sporen lebendig gezeichnet. Einer dieser ist die nämlichen Spore die wir in Fig. 6 gezeichnet haben.

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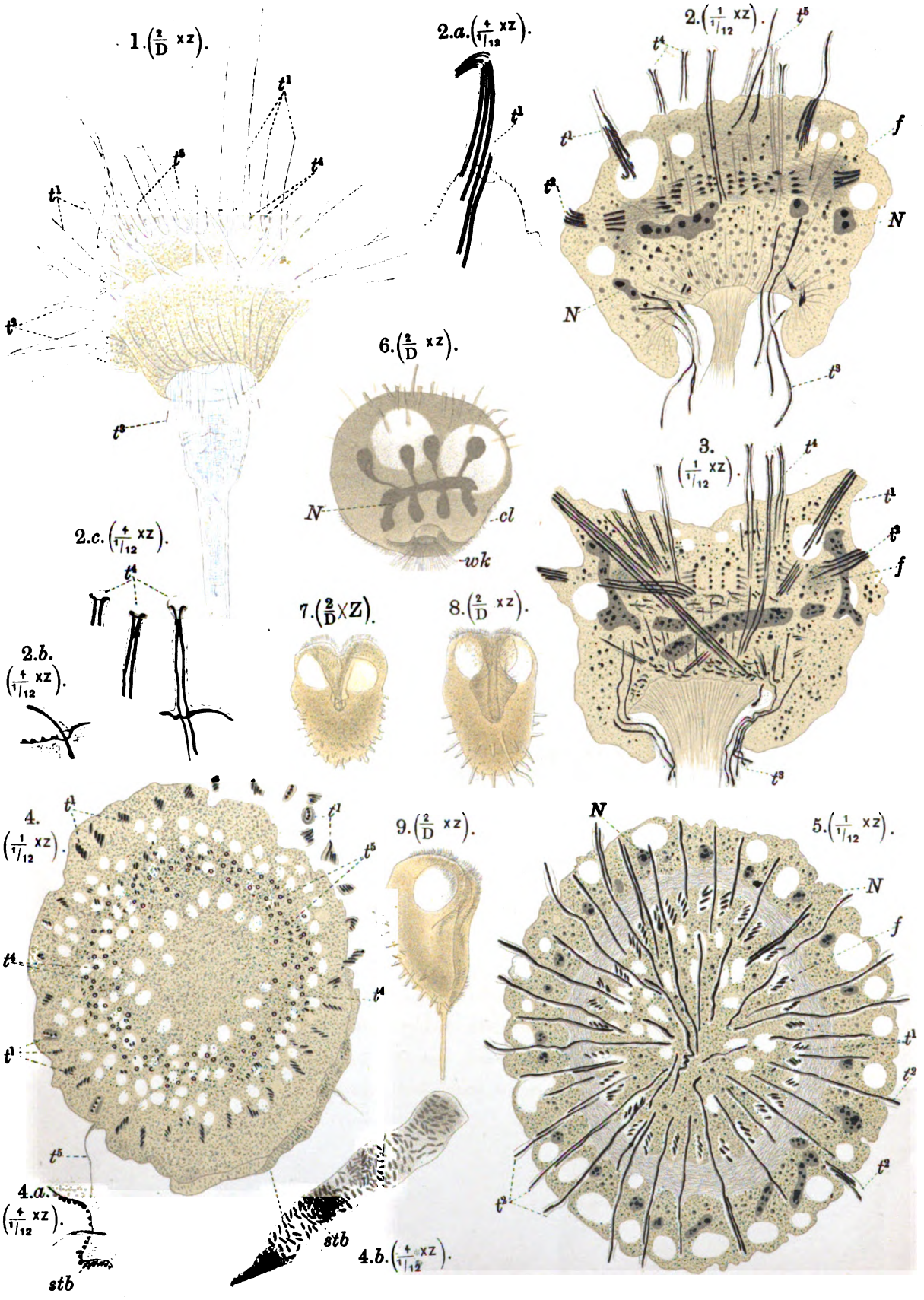

C. Ishikawa del.

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TAFEL XIII.

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Tafel XIII.

Ephelota spec. (?).

Sämmtliche Bilder sind mit einer Zeichencamera auf der Höhe des Objecttisches gezeichnet, und mit den Symbolen der dabei benutzten Oculare und Objective in Klammern beigefügt, wie $\left(\frac{2}{D}\times Z\right), \left(\frac{4}{1/12}\times Z\right)$ u. s. w. die als Zeiss' Objectiv D mit Ocular 2, Zeiss' Objectiv 1/12 Immersion mit Ocular 4 u. s. w. zu lesen ist. *n*, micronucleusähnlichen Gebilde; *N*, Macronucleus; *s*, Sporen; t^1, t^2, t^3, t^4 und t^5, Tentakeln; *wk*, Wimperkranz.

Fig. 10. Thierchen mit acht ganz reifen Sporen.

Fig. 11. Längsschnitt von einem anderen Individuum im Anfangsstadium der Sporenbildung. Sublimatessig-Präparat mit Orange-Hæmatoxylin gefärbt.

Fig. 12. Längsschnitt von einem solchen Thierchen wie es in Fig. 10 dargestellt ist. Drei von den Sporen werden in dem Schnitt angetroffen. Pikrinessig und Orange-Hæmatoxylin Präeparat.

Fig. 13. Längsschnitt von einem anderen Thierchen mit zwölf gestielten Sporen. Die Stielchen wurden aber nicht in dem Schnitt angetroffen. Flemming's Flüssigkeit mit Eisenlack-Hæmatoxylin; die Endoplasma-Granula sind weggelassen.

Fig. 14. Lebendes Exemplar mit sechszehn gestielten Sporen.

Fig. 14a. Dasselbe mit Pikrinessigsäure conserviert und mit Pikrocarmin gefärbt.

Fig. 15. Längsschnitt von einem anderen Individuum mit vier gestielten Sporen, ebenso behandelt wie bei Fig. 13.

Fig. 16. Gestielte Spore lebendig gezeichnet. Man beachte die protoplasmatische Verbindungsbrücke zwischen der Spore und dem Mutterkörper.

Fig. 17. Längsschnitt von einem Sprössling. Am freien Ende des Stieles sieht man Schmutz angeklebt. Flemming's Flüssigkeit mit Eisenlack-Hæmatoxylin.

Fig. 18. Längsschnitt von einem Individuum mit vier gestielten Sporen. Flemming's Flüssigkeit mit Eisenlack-Hæmatoxylin.

Fig. 19. Mittelgrosses Exemplar mit zwei gestielten Sporen. Man sieht drei parasitische Infusorien am Stiel eines Sprösslings. Diese dringen zuweilen in den Körper des Thieres ein und fressen den ganzen Inhalt.

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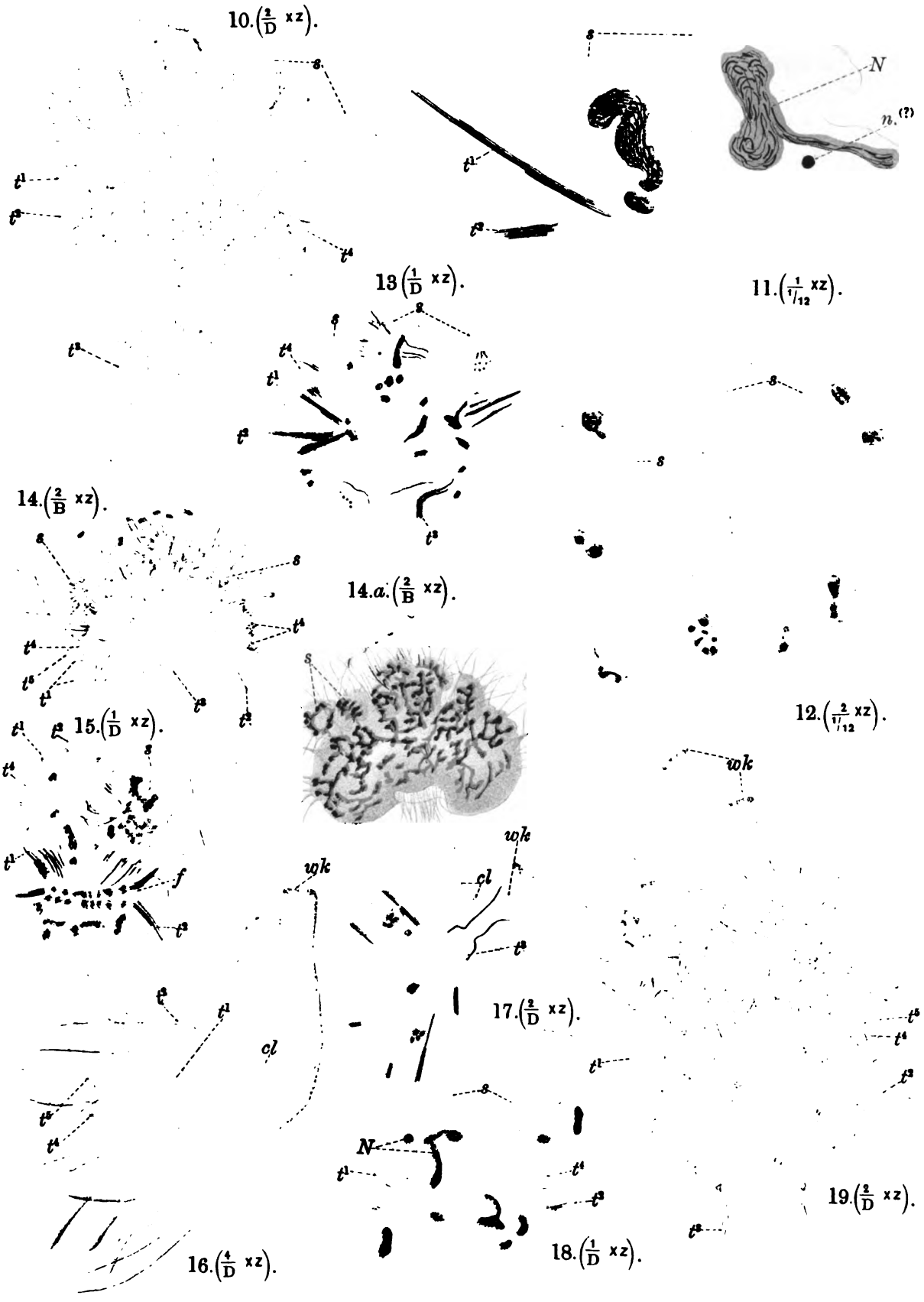

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Ueber das massenhafte Vorkommen von Eisenbacterien in den Thermen von Ikao.

Von

Manabu Miyoshi, *Rigakushi, Rigakuhakushi.*

Professor der Botanik an der naturwissenschaftlichen Facultät der Kaiserlichen Universität zu Tokio.

Die Umgebung des auf dem wilden Abhange des Futatsudake reizend gelegenen Badestädtchens Ikao, dessen eisenhaltige Thermalquelle wegen ihrer vortrefflichen Heilwirkungen sich seit einer geraumen Zeit einer bedeutenden Frequenz erfreut, ist für jeden Pflanzensammler eine reichliche botanische Fundgrube. Was aber uns hier besonders interessirt, ist das Vorkommen einer gänzlich aus Eisenbacterien bestehenden Schlammmasse, welche ich an der heissen Quelle auf Steinen oder Erde unter Einfluss des von Felsenspalten heraussickernden Thermalwassers sich verbreiten fand. Dieser Schlamm, welcher seiner vermeintlichen Heilkraft halber oft von Badegästen eingesammelt wird, unterscheidet sich von der übrigen bacterienfreien oder bacterienärmeren Eisenerde dadurch, dass der erstere homogen, leicht ockergelb gefärbt, wollig-schleimig aussieht, während die letztere von schmutzig brauner Farbe ist, fein körnig ist, sich leicht im Wasser suspendirt und daraus erst beim langen Stehen sich sedimentirt.

Dass der obenerwähnte Schlamm ausschliesslich aus Bacterienzellen besteht lehrt die mikroskopische Untersuchung sofort. Am bestens nimmt man eine möglichst kleine Probe des Schlammes, verbreitet sie gleichmässig auf einem Deckgläschen und trocknet über

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die Spiritusflamme. Bei einem so fixirten Praeparate sieht man eine unzählige Menge von gelblich gefärbten, fadenförmigen Zellen von ungleicher Länge, ca. $\frac{1}{2}$ bis 1 μ im Durchmesser, ziemlich geradlinig, auch verschiedentlich gebogen, sowohl einzeln als auch zu Bündeln vereinigt. Ausserdem befindet sich eine Anzahl von winzigen, stäbchenförmigen Zellen, die mit den fadenförmigen gemischt auftreten. Die Bacterien scheinen der *Leptothrix ochracea* Kütz. nahezustehen, dabei wage ich aber nicht ohne weitere Untersuchungen diese als neue Species zu betrachten.

Behandelt man solche Zellhaufen mit nur mässig starker Salzsäure so verschwindet nicht blos die Gelbfärbung, sondern auch der ganze Zellumriss spurlos, (der Zusatz einer Ferrocyankaliumlösung ruft dann die characteristische blaue Eisenreaktion hervor.) Ist die angewandte Säure aber noch weit schwächer und wirkt langsam von einer Seite des Praeparates dann entfärben die Zellen sich allmählich und lassen schwer sichtbare, hyaline Zellfragmente zurück, welche nach Färbung mit Fuchsinlösung deutlicher hervortreten. Unterbricht man indessen die Wirkung der Säure durch das Zuleiten von Wasser, so bleiben die Zellen oft zum Theil noch inkrustirt. In manchen Fällen verschwinden die von der Kruste befreiten Theile bald darauf und lassen die von der Säure nur wenig angegriffene, deshalb unvollständig aufgelöste Zellpartie zurück.

Aus diesem Verhalten gegen Salzsäure scheint mir zu folgen, dass das Eisenoxyd nicht nur auf der Zellwand aufgelagert, sondern auch in der Substanz derselben mehr oder weniger stark infiltrirt vorhanden sein muss.

Einer grobkörnigen Auflagerung entbehren die Zellwandungen vollständig ; bei genauer Untersuchung mittelst Zeiss'schen homog. Imm. $\frac{1}{12}$ und Ocular 4 fand ich nur äusserst feine Partikelchen die auf manchen Zellen aufsassen.

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Ob die Bacterienzellen noch plasmahaltig waren oder nicht, konnte ich der Winzigkeit derselben halber nicht konstatiren ; aber die starke Eiseninkrustation und sogar Infiltration, ferner das fragmentarische Auftreten der Zellen, machen es höchst wahrscheinlich, dass dieser Schlamm lediglich aus den Resten der Eisenbacterienzellen bestand.

Ich untersuchte auch Schlammproben die in der Nähe des oben geschilderten Bacterien-Schlammes gesammelt waren. Die ersteren sind schon mit blosem Auge von dem letzteren zu unterscheiden durch die mehr roth-braune Farbe und die sehr fein pulvrige Beschaffenheit. Mikroskopisch zeigt sich sofort ein ganz anderes Bild : in diesem, nämlich, besteht die Hauptmasse nicht aus Bacterienzellen allein, sondern auch aus gröberen oder feineren Körnchen von Eisenoxyd, die mit den ersteren bei verschiedenen Schlammproben in verschiedenen Mengenverhältnissen gemischt sind. Die Bacterienmassen enthalten sowohl faden- als auch stäbchenförmige Zellen, und auch noch eine Beimengung einer geringen Anzahl spirochaetenartiger Zellen, (einige Formen sind dem *Spirillum ferrugineum* De Toni ähnlich), die oft zu doppelten oder mehrfachen sogar verzweigten Bündeln vereinigt auftreten.

Andere gewöhnlichere Eisenbacterienarten z. B. *Crenothrix Kühniana* (Rabenh.) fand ich nicht in dem von mir gesammelten Materiale. Dagegen beobachtete ich mitunter wenn auch nur fragmentarisch, breitere Zellfäden mit Querwänden, die zweifelsohne eine Art von *Psichohormium* zu sein dürften. Weiter waren einige Diatomenzellen sehr oft in den weniger reinen Schlammproben vorhanden.

Alle jene Bacterienzellen sind mit Eisenoxyd dick überzogen und zeigen ähnliches Verhalben gegen Salzsäure wie es bei den oben beschriebenen Bacterien der Fall ist.

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Das Zustandekommen des reinen Bacterienschlammes ist von gewissem Interesse, da in diesem Falle das Eisenoxyd, welches bei der allmählichen Veränderung des Schlammes übrig bleibt, ausschliesslich aus dem Resten oder Fossilen der Organismen besteht.

Ich verzichte auf einen Erklärungsversuch betreffs der Art und Weise der Eisenauflagerung eventuell Infiltration, da die Entscheidung der Frage über den physiologischen resp. physikalischen Ursprung der Eisenscheide ohne fernere Studien nicht möglich ist.[1]) Nur mache ich hier besonders darauf aufmerksam, dass günstige Bedingungen zur Entstehung der Eisenbacterien-Schlammmasse unter Voraussetzung gewisser (direkter oder indirekter) Mitwirkung von Bacterien in den genannten Thermen vorhanden sind, da erstens, das Thermalwasser eine hinreichende Menge (nach einer Analyse 0,0214 g pro Liter) von doppelkohlensaurem Eisenoxydul enthält, zweitens, eine genügend hohe Wassertemperatur, die ich in oben erwähnten Stellen resp. 41°, 42°, 45,5°C fand, für das üppige Fortleben der Bacterien günstig ist.

d. 13. November 1896.

Botanisches Institut der Kaiserl. Universität zu Tokio.

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1) Hierüber liegen uns die bekannten Untersuchungen von Winogradsky, Botan. Ztg. 1888 p. 261. und von Molisch, Die Pflanze in ihrer Beziehung zum Eisen 1892 p. 60. vor.

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Studien über die Schwefelrasenbildung und die Schwefelbacterien der Thermen von Yumoto bei Nikko.

von

Manabu Miyoshi, *Rigakushi, Rigakuhakushi.*

Professor der Botanik an der naturwissenschaftlichen Facultät der Kaiserlichen Universität zu Tokio.

Mit Tafel XIV.

Einleitung.

Als ich im Monate August 1895 mich im Bad Yumoto bei Nikko aufhielt, wurde meine Aufmerksamkeit auf die auffallende Schwefelrasenbildung in diesen thermalen Gewässern gelenkt. An den Stellen, wo das heisse, stark nach Schwefelwasserstoff riechende Wasser aus den am Bergfusse des Yudaké reihenweise gelegenen Schwefelquellen heraussickert, bedecken sich Reservoirbasins, Wasserableiter, Pflanzenreste, Steinfragmente u. A. mit einem feinen Schwefelanflug in Form von bis mehrere Centimeter langen Franzen, Fetzen und Lappen, (Fig. 1), die ich der Kürze halber als Schwefelrasen bezeichne. Diese Rasenbildung kommt dadurch zu Stande, dass aus dem im Wasser reichlich enthaltenen Schwefelwasserstoff sich Schwefel abscheidet, der auf die Gallertsubstanz einer im heissen Wasserstrom vegetirenden Bacterienart sich absetzt und zum völligen Inkrustiren führt. Die Bacteriengallerte, welche mit

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der in den Schwefelthermen europäischer Länder vorkommenden „barégine" genannten Schleimmasse[1]) vielleicht identisch sein könnte, hat eine besondere Fähigkeit sich mit dem Schwefelmantel zu bekleiden und zwar so vollständig, dass der Process sich bis auf die dünnsten Fäden erstreckt.

Ausser diesen Gallertbacterien kommen auch gewöhnliche Formen von Schwefelbacterien vor, nämlich *Beggiatoen* und *Thiothrixen* (Winogradsky) (Fig. 13, 14), die in den lauwarmen Abflüssen des Quellwassers sowie in einem kleinen See Yugo, in den das noch mit H_2S beladene Wasser hineinfliesst, ihren Wohnsitz finden.

An rothen Schwefelbacterien fehlt es in der Umgebung von Yumoto auch nicht. In den Gräben und Tümpeln, die thermalen Abwässer empfangen, fand ich pfirsichblüthrothe Flecken welche ich bei der Untersuchung als *Chromatium Weissii* (Fig. 15) erkannte.

Um die Schwefelbacterien-Flora von Yumoto zu durchforschen und besonders die obenerwähnte Schwefelrasenbildung genauer zu beobachten, reiste ich in diesem Sommer wieder nach Yumoto, wo ich während eines mehrtägigen Aufenthaltes noch viele andere Arten, die bei dem früheren Besuch meine Aufmerksamkeit sich entzogen, fand und auch einige Versuche anstellte, die nur an den Fundorten selbst gemacht werden konnten.

Auch an anderen Thermen, die ich im letzten Jahre zu besichtigen die Gelegenheit hatte, fand ich eine ähnliche Schwefelrasenbildung. Da aber meine diesbezüglichen Studien fast ausschliesslich in Yumoto gemacht wurden, so theile ich im folgenden hauptsächlich die auf diesen Ort bezüglichen mit, während die Beobachtungen von anderen Orten nur nebensächlich erwähnt werden.

Da ich in dieser Abhandlung nur die Art und Weise des

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1) Certes und Garrigou, Comptes rendus. CIII. 1886. p. 107.

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Vorkommens von Schwefelrasen ausführlicher beschreibe, dagegen auf die Morphologie und Physiologie der Rasen bildenden Bacterien wenig Rücksicht nehme, möchte ich mir fernere Untersuchungen hierüber vorbehalten.

Die unten erwähnten physiologischen Studien an den rothen Schwefelbacterien wurden im botanischen Institute in Tokio ausgeführt.

Litteratur.

Biologische Untersuchungen des Thermalwassers sind im Deutschland, in der Schweiz, in Italien u.s.w. schon früher vielfach unternommen und das Vorkommen von niederen Algen und Bacterien in den heissen Wässern von Carlsbad, Landeck und anderen Thermen ist längst bekannt, wie besonders aus den Untersuchungen von Cohn[1]) zu ersehen ist.

Bekannt ist auch die Thatsache, dass diese im Thermalwasser vegetirenden Schizophyten und ihre Zoogloeenmassen von Schwefel, Kalk, Kieselsäure, Eisenoxyd und desgl. verschiedentlich inkrustirt vorkommen.

Was besonders die Schwefelthermen anbetrifft, so liegen uns nur vereinzelte Beobachtungen vor[2]) und überhaupt in der botanischen Litteratur findet sich meines Wissens nirgend eine genaue Angabe über die Schwefelrasenbildung. Certes und Garrigou[3]) berichten in ihren Untersuchungen über die Schwefelthermen von Luchon, dass bei einer Wassertemperatur von 50°C die schleimigen, „barégine" genannten Zoogloeenmassen auftreten, welche thatsächlich von ver-

1) Abhandl. d. schles. Gesells. f. vaterl. Cultur 1862; auch Flora 1862. p. 338. Jahresb. d. schles. Gesells. f. vaterl. Cultur 1874; botan. Sect. Nov. p. 32; und auch 1876. p. 115–118. Vergl. auch Hansgirg, Physiologische und algologische Studien 1887. p. 138.

2) z.B. Chr. Müller, Chemisch-physikalische Beschreibung der Thermen von Baden in der Schweiz 1870. p. 10 etc. Vergl. auch Loeffler's allerneuestes Werk, Das Wasser und die Mikroorganismen, (Handbuch der Hygiene I. Bd. 2. Abth. 1896. p. 701).

3) Comptes rendus. CIII. 1886. p. 703.

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schiedenen Organismen erzeugt sind. Anderseits theilen Peroncito und Valalda[1]) über die Muffe— oder Schwefelleberbildung in den Thermen von Valdieri in Piemont mit, dass bei 55°C die Muffe eine hochrothe Farbe annahm und keinen Organismus enthielt; und erst bei niederen Temperaturen, wenn die Muffe eine braune Farbe führt, die Gallerte bildenden Bacterien und *Leptothrix Valderia*, nebst grünnen Organismen auftraten. Da diese und andere Beobachtungen mit unserer Schwefelrasenbildung keine direkte Beziehung zu haben scheinen, so werde ich hier darauf nicht näher eingehen.

Die einschlägige Litteratur über die physiologisch merkwürdigen, von Winogradsky „Schwefelbacterien" genannten Organismen ist nicht besonders reich. Aeltere Beobachtungen finden wir in den Adhandlungen von Perty[2]), Ray Lankester[3]), Cohn[4]), Warming[5]), Engler[6]), Engelmann[7]), Zopf[8]) u. A. Aber eine gründliche und kritische Untersuchung verdanken wir erst Winogradsky[9]) der nicht blos durch die direkten Versuche die Physiologie

1) Notarisia II. 1887. p. 333–317. Referat in Jost, botan. Jahresb. 1887. I. p. 103. Mir war nur das Referat zugänglich.

2) Kleine Lebensformen 1852.

3) On a peach-coloured Bacterium, (Quarterly Journal of Microsc. Sc. Vol. XIII. New Series 1873). Further Observation on a peach or red-coloured Bacterium,—Bacterium rubescens, (Quart. Jour. of Microsc. Sc. Vol. XVI. New Series 1876).

4) Untersuchungen über Bacterien II, (Beitr. z. Biolog. d. Pflanzen Bd. I. Heft III. 1875. p. 156). In dieser Arbeit, sind seine früheren Untersuchungen über Schwefelbacterien berücksichtigt.

5) Om nogle ved Damarks Kyster levende Bacterier 1876.

6) Ueber die Pilz-Vegetation des weissen und todten Grundes in der Kieler Bucht, (IV. Ber. d. Commission 2 Unters. d. deutsch. Meeres in Kiel. Kiel 1883).

7) Over een niew voor licht gevoelig bacterium, (Proc. verb. k. Acad. v. Wettenschappen te Amsterdam. Afr. Naturk. Zittg. van 25 Maart 1882). Bacterium photometricum. Ein Beitrag zur vergleichenden Physiologie des Licht- und Farbensinnes, (Pflüger's Archiv. Bd. XXX. 1883. p. 95). Pfrüfung der Diathermanität einiger Medien mittelst Bacterium photometricum, (Dieselbe Zeitschrift, Bd. XXX. 1883. p. 125). Ueber Bacteriopurpurin und seine physiologische Bedeutung, (Dieselbe Zeitschrift, Bd. XLII. 1888.p. 183). Die Purpurbacterien und ihre Beziehungen zum Licht, (Bot. Ztg. 1888. p. 661).

8) Zur Morphologie der Spaltpflanzen 1882. p. 21 u. s. w.

9) Ueber Schwefelbacterien, (Bot. Ztg. 1887. p. 489). Beiträge zur Morphologie und Physiologie der Bacterien Heft I. Zur Morphologie und Physiologie der Schwefelbacterien 1888.

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der Organismen in Bezug auf Schwefelwasserstoff einwandfrei klarlegte, sondern durch eine Reihe langer, ununterbrochner, mikroskopischer Kulturen die Morphologie derselben festzustellen suchte.[1])

Die Wirkung des Lichtes auf die rothen Schwefelbacterien wurde von Engelmann[2]) bekanntlich besonders vom assimilationsphysiologischen Standpunkte aus zum Gegenstand eingehender Untersuchungen gemacht.

Das merkwürdige Verhalten derselben Organismen gegen wechselnde Sauerstoffspannungen geht auch aus der Untersuchung Winogradskys hervor; nur sind weitere Versuche nöthig, um festzustellen, ob die Organismen gegen den für sie so wichtigen Schwefelwasserstoff chemotaktisch reagiren und ob sie der chemischen Reizung durch andere Stoffe auch zugänglich sind.

Schwefelrasenbildung.

Die schönste und üppigste Schwefelrasenbildung fand ich bei einer am westlichen Ende von Yumoto gelegten heissen Quelle (Arayu) sowie in einem noch ca. 50 Schritten weiten Schwefelsumpf. Ein minder auffälliges Auftreten beobachtet man an der Quelle Tsurunoyu, dicht an der Strassenmündung von Yumoto. Wie schon erwähnt, breiten die Rasenmäntel sich an den Aussenwänden der Reservoirfässer und hölzerner Ableiter aus, aber auch selbst auf der Erde, über welche das Schwefelwasserstoff haltige, heisse Quell-

1) Nachdem Winogradsky im Jahre 1888 durch langdauernde mikroskopische Kulturversuche die früher bestrittene Formenkonstanz der Schwefelbacterien festgestellt hatte, ist seither kein wichtiger gegentheiliger Befund darüber bekannt geworden, bis in jüngster Zeit Zopf (Beiträge zur Physiologie und Morphologie niederer Organismen 5 Heft 1895. p. 37.) beweisen konnte, dass eine „regressive Entwickelung" bei den rothen Schwefelbacterien wirklich stattfindet. Ueber die Formenfrage vom gegenwärtigen wissenschaftlichen Standpunkte aus vergleiche man Hüppe, Naturwissenschaftliche Einführung in die Bakteriologie 1896. p. 14 u. 85; Flügge, Mikroorganismen 3. Aufl. 1896. Erster Theil p. 76 u. s. w.

2) l. c.

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wasser schnell abläuft. Zwei Bedingungen müssen betreffs der Rasenbildung erfüllt sein: erstens muss das Wasser stets fliessend sein, zweitens, die Wassertiefe nur sehr gering bleiben. Im tieferen, fliessenden Wasser bilden sich die Rasen nur auf den unmittelbar unter der Wasseroberfläche befindlichen Gegenständen und in stehenden Gewässern kommen sie überhaupt nicht vor.

Die Maximaltemperatur, bei welcher eine Rasenbildung stattfindet fand ich dicht bei der Arayu-Quelle zu 69.8°C. An den anderen Orten war die Wasserwärme resp. 68.2°, 67°, 66.1°, 61.5°, 59°, 58.2°, 56°, 55°, 52°, 51°C. Bei Bad Shibu und Bad Idsusan, wo ebenfalls die Schwefelrasenbildung vorkommt, beobachtete ich die Wassertemperatur zu resp. 68° und 60°. Die Temperaturgrenzen für die Rasenbildung liegen daher zwischen ca. 70°[1]) und 51°.

Was nun die Farbe der Schwefelrasen anbelangt, so ist sie je nach den Fundorten verschieden. In schnell fliessender Strömung sieht sie leicht gelb-weiss (Fig. 1), bis fast rein weiss aus, die gelbliche Färbung nimmt dagegen stetig zu bei langsamerer Strömung. So fand ich, an der Unterseite eines viereckigen, hölzernen Ableiters, aus welchem eine Menge des heissen Quellwassers auslief, das auf den Aussenwänden sich verbreitete, eine üppige, gelbliche Rasendecke, während die Innenwände derselben mit weissen Schwefelfilzen bekleidet waren.

Dieser Farbenunterschied beruht, wie die mikroskopische Untersuchung lehrte, auf der Art und Weise der Schwefelabsatzbildnng. Im langsamen Strome bilden sich feine, ziemlich grosse Krystalle,

1) Bekantlich vegetiren *Oscillarien* und *Leptothrisen* in heissen Thermalwässern deren Temperatur jedoch selten über 60°C. liegt. Doch sollen gewisse Schizophyceen nach einigen Beobachtern bei weit höheren Temperaturen von 80°–90°C. sogar 93° noch vorkommen. Vergl. Warming, Lehrbuch der ökologischen Pflanzengeographie. Uebersetzt von Knoblauch. 1896. p. 157. Die Angabe der älteren, diesbezüglichen Beobachtungen findet man in Pfeffers Pflanzenphysiologie Bd. II. 1881. p. 432 u. s. w. Vergl. auch Kerner von Marilaun, Pflanzenleben Bd. I. 1888. p. 517.

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zumeist in rhombischen Octaëdern oder auch in monoklinen Prismen, die das Licht stark brechen und die eigenthümliche Farbe des Elementes darstellen (Fig. 2, 5); hingegen im schnell fliessenden Wasser wird der Schwefel in winzigen, amorphen Theilchen mit Beimengung unvollkommen ausgebildeter Prismen abgesetzt; diese kleinen Körnchen reflectiren das Licht stark und verleihen dem Ganzen eine mehr weisse Färbung (Fig. 3, 4). Zwischen diesen beiden Extremen treten aber alle Uebergangsstadien auf. Die grauweissen Rasen, die man oft finden kann, sind nur durch Schmutz verunreinigte, sonst normale Rasen; die Beschmutzung geschieht besonders bei heftigem Regen, welcher bisweilen eine prachtvolle Rasendecke vernichtet.

Die Länge der Rasen ist wieder von der Schnelligkeit des Stromes abhängig. Die gewöhnliche Länge in ziemlich schnell fliessendem Wasser beträgt ca. 3 bis 5 cm. Sie bleiben kurz ca. 0.5 bis 2 cm bei langsamer Strömung und erreichen in schnellen Bächlein mehr als 20 cm. Besonders elegant gestalten sich diese langen, gelbweissen Schwelfelrasen, wenn sie der Stromsrichtung folgend, auf zahlreichen, einzelnen Fasern zitternde Bewegungen zeigen.

Die Rasen sind ursprünglich durch die schleimige Bacteriengallerte auf Holz, Steinen oder anderen submersen, fremden Gegenständen, so fest angeheftet, dass sie trotz des in Folge der Volumenvergrösserung immer zunehmenden Zuges von den Ansatzstellen nicht leicht fortgerissen werden können.

Untersucht man ein kleines Büschel Schwefelrasen, das durch sorgfältiges Präpariren mittelst Nadelspitzen in feine, einzelne Fasern getrennt werden kann, so sieht man, dass an der Peripherie der aus Schwefelkryställchen und schwefelkörnchen bestehenden Hauptstückes, eine unzählige Menge von sensenförmigen Bacterienzellen anhaftet

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(Fig. 6). Bei genauerer Untersuchung aber überzeugt man sich, das die Matrix der Rasen ursprünglich von einer Bacteriengallerte gebildet war und die Schwefelauflagerung nachträglich stattgefunden hat.

Dies kann man leicht beweisen, wenn man einen solchen Büschel auf dem Objecträger trocknen lässt und dann etwas Schwefelkohlenstoff zusetzt.[1]) In dem so behandelten Praeparate wird die Schwefelauflagerung mehr oder weniger vollständig aufgelöst und die übrige Gallertmasse mit Bacterienzellen tritt bei der Färbung mit Jod scharf hervor. Am besten aber wähle man zur Untersuchung solche Rasenstücke, welche erst geringe Schwefelinkrustation zeigen (Fig. 2, 6). An derartigen Exemplaren, die nur an bedeckter, vor freiem Luftzutritt mehr oder weniger geschützter Lage zu finden sind, sieht man gleich, ohne eine weitere Behandlung, den centralen, farblosen Gallerttheil mit den darin eingebettenen Bacterien und die äusserlich stellenweise angelagerten Schwefelkryställchen.

Dass die einzelnen Bacterienzellen mittelst der gemeinsamen, schleimigen Gallerte zusammen gehalten sind, kann man unter dem Mikroskop demonstriren, indem man entweder gelinde auf die Deckgläschen stösst oder mittelst einer Fliesspapierstreifchen einen raschen Wasserstrom durchleitet ; dann machen die Bacterienzellen jedesmal zitternde Bewegungen ohne jedoch dadurch losgerissen zu werden.

Die Gallerte hat eine schleimige, etwas zähflüssige Konsistenz und zieht sich zu einem Kügelchen zusammen wenn man sie aus dem Wasser herausnimmt. Sie ist z. Th. löslich in conc. Kalilauge und in Salzsäure ; bleibt in kochendem Wasser scheinbar unverändert ; färbt sich mit Jod gelb, mit Jod und Schwefelsäure ziegelroth bis braun, mit Corallin hochroth, mit Hanstein's Anilingemisch scharlachroth, mit Congo roth : kurz, sie giebt alle characteristischen

1) Vergl. Cohn, Untersuchungen über Bacterien II, (Beitr. z. Biolog. d. Pflanzen. Bd. I. Heft III. 1875. p. 179).

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Farbenreaetionen der Schleimsubstanz.[1])

Die schleimige Gallerte enthält hauptsächlich cylindrische, sensenförmig gebogene, oder vibrionenartige, an beiden Seiten abgerundete, farblose Zellen von verschiedenen Dimensionen, 6 bis 28 μ in Länge, (mittlere Grösse 20 μ lang und 1,4 μ breit), und mitunter auch fadenförmig gestreckte *Leptothrix*-ähnliche Zellen.

Bei Zimmertemperatur untersucht führten einige von den vibrionenartigen Zellen schwingende und umdrehende Bewegungen aus, welche bei den grösseren Zellen langsamer und bei den kleineren lebhafter zum Vorschein kamen. Nach der Anwendung von A. Fischers[2]) Geiselfärbungsmethode konnte ich bei verschiedenen Individuen dicht um den Körper ausgewachsene Geiseln beobachten.

Ausserdem fand ich in den Gallertmassen noch folgende Zellarten, die jedoch in den an verschiedenen Standorten gesammelten Proben in verschiedenen Mengenverhältnissen vorhanden waren :— sehr kleine, stäbchenartige Zellen, die oft so massenhaft auftraten, dass das Ganze wie eine *Zoogloea Termo* Cohn[3]) aussah ; dünne, lange, Fäden öfters in einer mässigen Anzahl ; winzige spirochaetenartige Zellen, die in einigen Gallertproben zerstreut gefunden wurden.

Die Zellformen in den schleimigen Gallerten sind daher von heterogenem Character und das ganze Bild ähnelt etwa der *Zoogloea ramigera* Itzigsohn, die Zopf[4]) bekanntlich auf Grund seiner Untersuchungen für einen besonderen Zoogloeenzustand von *Cladothrix dichotoma* Cohn hält. Ich machte bisher noch keine genauere Studien über die Entwickelungsgeschichte unserer Bacterien und

1) Behrens, Tabellen zum Gebrauch bei mikroskopischen Arbeiten. 2te. Aufl. 1892. p. 145.

2) Untersuchungen über Bakterien. Separat-Abdruck a. d. Jahrb. f. wiss. Bot. Bd. XXVII. 1894. p. 81.

3) Beiträge zur Biologie der Pflanzen Bd. I. Heft 2. 1874. Taf. III. Fig. 9.

4) Zur Morphologie der Spaltpflanzen 1882. p. 19. Taf. III. Fig. 13, 14, 15.

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eventuell die Bildung des Gallertkörpers und so mag es noch dahingestellt bleiben, ob die Gallertmasse thatsächlich auch eine derartige Zoogloea sei, wie Zopf angiebt, und somit alle obenerwähnten Zellformen in einem genetischen Zusammenhange stehen, oder ob sie im Gegentheil den Wohnsitz einer Anzahl heterogener Spaltpilze vorstelle. Diese und noch andere morphologischen Fragen sind die Aufgabe weiterer Untersuchungen, die ich bei nächster Gelegenheit aufzunehmen gedenke.

Irgend einen fremden Einschluss enthalten unsere sensenförmigen Bacterienzellen nicht. Nur kommen einzelne, aber wenige Individuen vor, welche äusserlich mit Schwefelkörnchen versehen sind (Fig. 6, 7, 8). Die letzteren sind kugelich, verhältnissmässig gross, fest angehaftend, von eigenartigem, schwarzem Glanz und einreihig in fast gleicher Entfernung von einander getrennt. Diese Schwefelkörnchen scheinen auf den ersten Blick im Zellinneren aufgespeichert zu sein und es ist sogar in manchen Fällen nicht leicht, ihre wahre Lage zu beurtheilen.[1]) Jedoch beobachtete ich bis jetzt keinen sicheren Fall, wo die Körnchen wirklich im Zellinneren gelegen wären. Sollte der Fall vorkommen, so könnte man aus dem nur sporadischen Auftreten der schwefelhaltigen Individuen unseren Organismus als eine Art von Schwefelbacterien doch noch nicht betrachten. Wie dem auch sei, ist nur so viel wahrscheinlich, dass die Zufuhr von H_2S zur Lebensthätigkeit nothwendig ist, da ich den betreffenden Organismus bisher in keinen anderen als H_2S-haltigen, heissen Quellen gefunden habe.

Hier taucht die Vermuthung auf, dass unsere Bacterien vielleicht den H_2S *direkt* zu Schwefelsäure oxidiren, ohne erst Schwefel abzuscheiden. Denn, es ist ja wohl begreiflich, dass einerseits, die hohe Energie des Bacterienprotoplasmas, welches bei so ungewöhnlichen

1) Vergl. Winogradsky, ü. Schwefelbact. l.c. p. 588-589.

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Temperaturen wie 51° bis ca. 70°C thätig ist, anderseits, der Ueberschuss an Sauerstoff, von dem die Bacterienrasen nur durch eine dünne Wasserschicht getrennt sind, den genannten Oxidationsprocess leicht zu Stande bringen könnten. Somit würde in derselben Zeit hier noch weit mehr Energie gewonnen als bei jenen Schwefelbacterien (z.B. *Beggiatoa*,) die bei mangelhaftem O-Zutritt aus dem H_2S zuerst Schwefel abscheiden, um dann später denselben zu SO_4H_2 oxidiren.[1])

Der Umstand, dass unsere Bacterien nur in fliessendem Wasser gedeihen, ist vielleicht z. Th. darauf zurückzuführen, dass so die in den Zellen entstehende SO_4H_2 viel rascher neutralisirt werden kann, durch die im Wasser vorhandenen Bicarbonate[2]), was wegen der Giftwirkung der freien Säure unbedingtes Erforderniss ist.

Eine andere Art von Gallertebacterien kommt im Bad Idsusan vor, wo ich an der Aussenseite eines Centralreservoirs des Thermalwassers ebenfalls die Schwefelrasenbildung beobachtete. Die Rasen sind weissgelb und 1 bis 2 cm. lang (Fig. 9) ; die sensenförmigen Zellen sind aber äusserst klein, nur ca. 4 μ lang und 0,3 μ breit, (Fig. 10, 11). Ob bei ihnen Schwefelkörnchen auch ausnahmsweise aufgelagert werden, konnte ich der Kleinheit wegen nicht entscheiden.

In den weniger heissen, aber stark sauren Schwefelthermen vegetirte eine echte *Leptothrix*art, welche eine lockere Schwefel-

1) Die direkte Oxidation von H_2S zu Schwefelsäure geht unter allen Umständen leichter vor sich, als die des fein vertheilten Schwefels, vorausgesetzt, dass genügende Sauerstoffmengen vorhanden sind.

2) Das Gosho-no-Yu Wasser (Yumoto) enthält in 1 Liter 0,0624 gr. von Calciumbicarbonat, („Nippon Kōsenshi" oder „Die Mineralwasser-Quellen Japans." Eine amtliche Mittheilung vom hygienischen Bureau des Ministeriums des Inneren Bd. II, 1885. p. 3.)

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auflagerung auf ihren Zellwänden hat, aber wegen des Mangels an Gallertsubstanz keine Schwefelrasenbildung veranlasst.[1])

Um nun die Art und Weise der Schwefelauflagerung auf der Bacteriengallerte zu studiren, suchte ich zuerst vergeblich die schwefelfreie Gallertmasse, die ich endlich in einem mit Brettern bedeckten Ableiter des Arayu-Wassers fand. In dem oberen Theile der Wasserleitung innerhalb einer Strecke von etwa 2 Meter, wo ein beschränkter Luftzutritt gestattet war, sah ich im schnell fliessenden Wasser von 69° bis 69,8°C eine reichliche Entwickelung der farblosen Gallertbacterien, die dicht an der Innenwand des hölzernen Ableiters anhafteten. Weiter unten, wo die Wassertemperatur unter 68° sinkt, beginnt schon stellenweise Schwefelabscheidung, welche besonders auf den der Wasseroberfläche näheren Gallertmassen stattfand. Eine vollkommene Schwefelrasenbildung geschah aber nicht eher, als etwa in einer vom Reservoir 5 Meter weit entfernten Stelle.

Die oben erwähnte scheinbar farblose Gallerte enthielt doch, nach meinen mikroskopischen Untersuchungen, winzige Schwefelkörner, die vereinzelt auf der Oberfläche sassen. Das Unterbleiben des massen-

1) Im Bad Kusazu, dessen Thermalwasser durch seinen starken Säuregehalt bekannt ist, [freie Schwefelsäure 1,3 bis 2,1 gr. pro Liter, freie Salzsäure 0,8 gr., Schwefelwasserstoff nur 0,004 gr. („ Die Mineralwassel-Quellen Japans," l.c. Bd. II. 1885. p. 4,9 u. s. w.)], kommt eine üppige Vegetation einer *Leptothrix*art bei Wassertemperaturen von ca. 25° bis 53°C vor. Ihre schlanken, dünnen, oft mehreren Decimeter langen dicht mit feinem Schwefelpulver bedeckten Fäden stehen im ruhigen oder nur langsam fliessenden Wasser fast senkrecht oder etwas schräg nach oben und führen sanfte Bewegungen mit der Stromrichtung aus. Der Schwefel ist auf die Zellwandung nur locker aufgelagert und wird durch eine schwache Erschütterung losgerissen. Die Fäden sind unverzweigt, cylindrisch und gleichmässig dick, von nur 0,7 μ im Durchmesser; das basale Ende, d. h. die Ansatzstelle ist nur wenig verdickt; mit Jod gefärbt tritt die Pseudogliederung hervor. Diese Art nenne ich *Leptothrix sulphurea*. Später fand ich dieselbe auch in dem stark sauren Thermalwasser (22° bis 42°C) in den Solfatarengegenden von Hakone. Dagegen traf ich diese nicht in Yumoto (Nikko) dessen Quellwasser nur sehr schwach sauer reagirt, welches aber wegen des reichen Gehaltes an H_2S (etwa 0,04 gr. pro Liter, also 10 mal mehr als Kusazu-Wasser), ein üppiges Auftreten einer Schwefelbacterienflora ermöglicht. Umgekehrt sucht man diese Organismen vergeblich in Kusazu oder den Hakone-Solfataren, deren Gewässer zu wenig H_2S und zu viel Säure enthalten.

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haften Incrustirens hat seinen Grund in dem mangelhaften Luftzutritt, wodurch die Oxidation des H_2S erschwert wurde. Denn einen deutlichen Niederschlag von Schwefel konnte ich beobachten, wenn ich eine Flasche mit demselben Wasser füllte und ohne einen Deckel im Ableiter selbst stehen liess. Weitere Versuche führte ich folgendermassen aus :—

1) Ein Büschel der schwefelfreien Gallerte von der oben genannten Stelle wurde bei derselben Temperatur 69°C in einen anderen Theil der Wasserleitung übergeführt, welcher offen und der Luft exponirt war. Hier war nach einer halben Stunde eine deutliche Schwefelauflagerung wahrzunehmen.

2) Einige andere Büschel derartiger schwefelfreier Gallerte wurden in eine offene Quelle von 59°C in den Schwefelsumpf gebracht und einem schnellen Wasserstrom ausgesetzt. Nach $\frac{1}{4}$ Stunde schon nahmen die Gallertmassen ein milchweisses Aussehen an. Die Schwefelausscheidung nahm stetig zu und nach einer Stunde waren die Gallertbüschel so reichlich incrustirt und von der ursprünglichen flockigen Gestalt so verschieden, dass ich ihn von den nebenstehenden Schwefelrasen nicht mehr unterscheiden konnte.

3) Eine Anzahl feiner Zwirnfäden wurden resp. mit Stärkekleister, halb coagulirtem Eiweiss, conc. Gelatinelösung oder dickem Leim bestrichen und zugleich an derselben Stelle wie Versuch 2 während $\frac{1}{2}$ Stunde schweben lassen. Auf allen diesen Objecten fand eine nur sehr unbedeutende Schwefelabscheidung statt, keineswegs mit der reichlichen, wie sie auf der Bacteriengallerte selbst (Versuch 1) stattfand, vergleichbar.

4) Papier-oder Zwirnfasern die nicht mit einer Gallertsubstanz befeuchtet waren, zeigten nach 1 Stunde nur spärliche Schwefelkörner.

Aus diesen Versuchen sehen wir, dass die Fähigkeit, sich mit dem Schwefelmantel zu umkleiden, wenigstens bei allen von mir benutzten

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Gallertarten weit zurücksteht hinter derjenigen der Bacteriengallerte ; diese merkwürdige Schwefelanhaftung muss daher durch die specifischen Eigenschaften der Gallertsubstanz zu Stande kommen.

Diese Schwefelincrustirung muss an den offenen Stellen von Anfang der Gallertentstehung an von Statten gehen. Irgend eine nachherige Vergrösserung der ganzen Gallertmasse hat auch immer eine entsprechende Volumenzunahme des Schwefelrasens zur Folge. Und da diese Schwefelbedeckung sich bis auf die feinsten einzelnen Fasern erstreckt, so sucht man vergeblich in einem unbedeckten Quellabflusse ursprüngliche schwefelfreie Gallerte.

Dass diese dichte Schwefelumkleidung dem Bacterienleben nachtheilig sei, ist nicht wahrscheinlich, da die Zufuhr der nöthigen Nahrungsstoffe desshalb noch nicht erschwert wird ; vielmehr könnte der Vorgang von Vortheil sein, insofern als die zarten Gallertemassen dadurch gegen manche äussere Einflüsse geschützt werden.

Anders ist es bei den im bedeckten Raume vegetirenden Rasen, wie oben erwähnt. Hier bleiben sie, so lange die ursprüngliche Bedingung erhalten ist, von einer Schwefelincrustation verschont.

Farblose Schwefelbacterien.

Nächstfolgende, gemeinsame Schwefelbacterienarten kommen im Thermalabwasser von Yumoto reichlich vor.

Thiothrix nivea (Rabenh.) Winog. [Beitr. p. 39. Taf. I. Eig. 7, 9, 10.] Im Schwefelsumpf u. A. (20° bis 29°C), bildet grauweisse mit Schwefelkörnchen beladene Büschel auf im Wasser befindlichen Gegenständen, besonders auf Pflanzenresten.

Thiothrix nivea (Rabenh.) Wing. nov. var. *verticillata*. Fig. 13. Zellen fast gleichmässig dick, 2, 5 bis 3 μ. Von den Ansatzstellen gesellig und strahlig angewachsen, sitzen neue Zellen wiederum

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strahlig an verschiedenen Stellen eines ausgewachsenen Eadens, so dass, ein characteristisches, wirbelförniges Aussehen resultirt. Im Yugo-See (22°C), auf Moosblättern, Algenfäden etc.

Thiothrix tenuis Winog. [Beitr. p. 40. Taf. I. Fig. 8, 11.] Fig. 14. Zusammen mit *Thiothrix nivea* in einem Abfluss (29°C) von Thermalwasser.

Beggiatoa alba (Vouch.) Trev. [Winog. Beitr. p. 23. Taf. I. Fig. 1.] In Gräben und Tümpeln (16°–21°C.)

Rothe Schwefelbacterien.

Die Bodenfläche des Schwefelsumpfes zu Yumoto ist verschieden gefärbt. Neben dem prachtvollen weissen Schwefelrasenmantel kommt die blaugrünliche Farbe einer üppigen Vegetation von Oscillarien sowie anderer Algen und ein braungelber Schlamm einer Diatomeenflora zum Vorschein. Ausser diesen bunten Färbung kommen auch hie und da leicht rothe bis carmoisinrothe Flecken vor, die eine bedeutende Ausdehung haben. Diese verrathen auf den ersten Blick die Wohnsitze der *Chromatium*arten, welche bei genauerem Suchen an noch vielen anderen Orten bei Yumoto gefunden werden.

Folgende Arten rother Schwefelbacterien kommen vor: —

Chromatium Weissii Perty. [Winog. Beitr. p. 97. Taf. IV. Fig. 1.] Fig. 15. Am häufigsten. Im August 1895 fand ich eine grosse Colonie auf dem dicht hinter dem Jizai-Yu gelegenen Grabenboden, dessen Oberfläche mit der schönen, rothen Haut fast bedeckt war. Im August dieses Jahres aber fand ich an derselben Stelle keine Spur mehr davon, dagegen erschien es in einem anderen Graben, wo es im Jahre vorher nicht zu sehen war. Die Wassertemperatur der Fundorte fand ich von 23° bis 35°C.

Chromatium minus Winog. [Beitr. p. 99. Taf. IV. Fig. 5.]

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Fig. 16. Auf dem Boden des Schwefelsumpfes. 26°C.

Chromatium vinosum (Cohn) Winog. [Beitr. p. 99. Taf. IV. Fig. 6, 7.] Fig. 17. Fundorte wie vorige. 29° bis 42°C.

Chromatium minutissimum Winog. [Beitr. p. 100. Taf. IV. Fig. 8.] Fig. 18. Fundorte wie vorige. 28°C.

Thioderma roseum nov. Gen. et Sp.[1]) Fig. 19. Zellen sphaeroidisch' 2.5 μ lang, 1.5 μ breit, leicht roth gefärbt, mit kleinen, winzigen Schwefelkörnchen. Zu dünnen, abnehmbaren matt purpurrothen Häutchen vereinigt. Schwärmfähig. Auf Grashalmen, Grasblättern etc. in einem feuchten Boden bei Jizai-Yu. 27°C.

Alle oben erwähnten Arten von 1 bis 4 fand ich selten für sich allein, meistens gemischt.

Zur Physiologie des Chromatium Weissii.

Wir behandeln hier hauptsächlich die chemotaktische Reizbarkeit, die wir bei unserem Organismus experimentell zu beweisen suchten. Bevor wir aber zur Schilderung der Resultate schreiten, mag noch Einiges über die Kultivirung gesagt werden.

Ich nahm am 26. August 1895 ein Quantum des *Chromatium*-Schwärmer haltenden, rothen Wassers nebst einer kleinen Menge von Schlamm aus demselben Fundorte mit nach Tokio. Am 31. August als das Material untersucht wurde, war die ursprüngliche, schöne, rothe Färbung fast verschwunden und der graubräunliche Bodensatz hatte nur einzelne noch lebendige Exemplare. Um nun eine Reihe von Kulturen anzustellen, wählte ich 3 je 500 ccm haltende, flache Gefässe mit weiter Oeffnung. In die erste kam nur der Schlamm, in die zweite noch ein geringer Zusatz von Gyps, die dritte enthielt statt Schlamm Schwefelrasen (Bacteriengallerte). Nachdem alle 3 Gefässe einen

1) Eine andere Art dieser Gattung trat im meinen Kulturbassin für Schwefelbacterien auf. Siehe den Anhang zu dieser Arbeit.

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Zusatz vom ursprünglichen *Chromatium*-haltenden Wasser erhalten hatten, wurden sie mit Deckeln versehen und in einem dunklen Schrank aufbewahrt. Am 17. September war das *Chromatium* in der Flasche I zu voller, neuer Entwickelung gelangt, indem das Gefässwasser mit rothen, wolkigen Massen getrübt war. Die Flasche III hatte nur an bestimmten Stellen der Schwefelrasenmasse lebhaft gefärbte Flecken, die sich von den übrigen, weissgelben Theilen scharf unterschieden. In der Flasche II war es noch zu keiner makroskopisch sichtbaren Entwickelung gekommen.

Während in No. I und III keine Vermehrung später beobachtet wurde, und die rothe Färbung allmälig verschwand, kam am 8. November in No. II ein plötzliches Auftreten der rothen Färbung oberhalb des Schlammes, besonders aber an der inneren Wandung der Flasche, zu Stande. Das Wasser roch deutlich nach H_2S und bräunte das Bleipapier. Die Entwickelung der rothen Organismen schritt täglich fort bis zur Mitte des Monats und sank dann wieder allmälig, obgleich die Schwärmer nach 5 Monaten nicht gänzlich abgestorben waren[1]). Während dieser Zeit beobachtete ich, dass die ungünstigen Bedingungen wie Nahrungs—sowie H_2S—Mangel und sonstigen Schädlichkeiten ausgesetzten Zellen in den Schlamm sanken, aber besonders massenhaft sich zwischen dem Boden und der Flaschenwandung ansammelten, wo sie zur Bildung einer mattrothen Haut führten.

Die Zellen gingen auf die Weise nicht gleich zu Grunde, sondern behielten noch eine Zeit lang Lebensfähigkeit, denn es gelang mir oft, ein aus solchen unbeweglichen, jedoch noch leicht-roth gefärbten Zellen bestehendes Häutchen unter Zufuhr von schwachem H_2S-Wasser und nach kurzem Einfluss des vom Mikroskopspiegel reflektirten

1) Zeitweilige Zufuhr vom schwachen H_2S-Strom ins Kulturwasser schien vortheilhaft zu sein, dagegen die Anwendung stärkeren H_2S-Wassers wurde als schädlich erkannt.

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Lichtes wieder zum Schwärmzustande auslösen zu können,—die bisher todstill gebliebenen Zellen begannen zuerst an den Rändern des Haufens eine zitternde Bewegung auszuführen, welche aber allmälig zu einer kräftigen, zuckenden übergingen und schliesslich rissen sie sich von der Masse los. Diese Erscheinung dauerte so lange, bis ein grosser Theil des Häutchens nach und nach schwärmfähig wurde. Diese Versuche gelangen nur bei denjenigen Individuen, welche sich noch roth färbten und einige Schwefeleinschlüsse im Zellinneren führten, dagegen war es vergeblich, bei einem verblassten, schwefelfreien Exemplar Lebenserscheinungen hervorzurufen.[1])

Da Schwefelwasserstoff beim Gedeihen der Schwefelbacterien, wie die Untersuchungen von Winogradsky bewiesen haben, eine wichtige Rolle spielt, so drängt sich die Frage auf, ob unsere rothen Bacterien überhaupt durch H_2S chemotaktisch reizbar seien und wie sich diese gegen noch manche andere Stoffe verhalten. Es wurde desshalb eine Reihe von Versuchen im verflossenen Herbst angestellt und zwar mit den Proben, welche aus der oben geschilderten Kultur II stammten. Zur Anwendung kam die bekannte Pfeffer'sche Capillarmethode.[2]) Der Durchmesser der Capillare an der Mündung war ca. 0,05 mm und die Länge derselben ca. 1 cm. Zum Ueberführen des Materials benutzte ich eine kleine Glaspippette, mit welcher ich eine beliebige Menge der rothen Schwämer direkt aus dem Kulturbassin einsaugen konnte. Wegen der auffallenden Farbe sowie der bedeutenden Grösse einzelner

1) Dass die Zellen auch einem Wassermangel in gewissem Grade widerstehen zeigt der folgende Versuch:—Ich liess einige lebhaft bewegliche Individuen auf einem Objectträger ca. 1 Stunde lang allmälig eintrocknen. Einige Exemplare verloren dabei ihren Farbstoff und waren mehr oder weniger geschrumpft, während andere noch die ursprüngliche Farbe behielten und nur wenig abgerundet wurden, dabei zeigten sie keine Spur von Bewegung. Nach der Herstellung des Wasserstroms begannen jedoch die noch roth gefärbten Zellen wieder zu schwärmen.

2) Locomotorische Richtungsbewegungen durch chemische Reize, (Unters. a. d. botan. Inst. z. Tübingen Bd. I. Heft III. 1884. p. 367.)

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Bacterien konnte man das Verhalten der ganzen Schwärmer gegen den eingeführten Stoff schon bei einer schwachen Vergrösserung deutlich erkennen.

Schwefelwasserstoffwasser.

A) Eine schwache Lösung. Das Bleipapier färbte sich leichtbraun :—

a) Die Schwärmer befanden sich in der ursprünglichen Flüssigkeit :—

Zuerst eine starke Ansammlung an der Capillarmündung und dann folgte das Eindringen in das Capillarinnere. Die Schwärmer verliessen aber die Capillare wieder und kamen nach Aussen.

b) Die Schwärmer waren in dem Leitungswasser :—

Ein momentanes Ansammeln um die Mündung und bald darauffolgendes Eindringen ins Innere der Capillare. Ein sofortiges Herauskommen der eingedrungenen wie bei (a) resultirte dabei nicht; sie drangen im Gegentheil immer weiter hinein. Wahrscheinlich wirkte hier H_2S stärker, da die Schwärmer vorher im gewöhnlichen Wasser sich befanden und dadurch vielleicht an einem „Schwefelhunger“ litten.

B) Eine stärkere Lösung. Das Bleipapier färbte sich bräunlich-schwarz :—

Die Schwärmer waren in der ursprünglichen Flüssigkeit.[1]) Zuerst eine Ansammlung in gewisser Entferung von der Capillarmündung. Die Masse rückte aber allmälig vorwärts und nach ½ Stunde bildete sich ein dichtes

1) In allen unten beschriebenen Fällen, wenn nicht besonders erwähnt, befanden sich die Versuchsorganismen stets in der Kulturflüssigkeit.

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Gedränge um die Mündung sowie in dem ersten Theil der Capillare. Langsam, doch immer vorwärts, gingen die Schwärmer und zwar mit lebhafterer Bewegung im Inneren und kamen 6 Stunden nach Beginn des Versuches bis zu einer in der Capillare bereits vorhandenen Luftblase. Ich wiederholte den Versuch mit H_2S-Wasser und gelangte zu ähnlichen, positiven Resultaten. Betont muss aber werden, dass eine unverkennbare Schwankung der Reizempfänglichkeit seitens des Organismus in diesem Falle sowie in anderem existirte, eine Schwankung, welche bei zwei andern gleichen Versuchen mit ebenso starkem H_2S-Wasser zu widersprechenden Resultaten führte.

Kaliumnitrat.[1])

0,3%. Eine starke, momentane Ansammlung um und in dem oberen Raum der Capillare (Fig. 20.) Während die dichten Schwärmer langsam nach dem Capillarinneren vordrangen, steuerten andere Individuen von verschiedenen Richtungen nach der Reizquelle hin und dies dauerte einige Minuten lang, bis in der Aussenflüssigkeit überhaupt nur eine geringe Anzahl mehr von ihnen gefunden werden konnte. Eine Stunde nach Beginn des Versuches bildeten die eingedrungenen eine dichte, ca. 1,5 mm lange Zone, welche immer weiter vorwärts rückte, bis sie schliesslich nach 3 Stunden eine von der Capillarmündung 6 mm weit gelegene Luftblase berührte (Fig. 21.) In einer feuchten Kammer aufbewahrt blieben die Schwärmer

1) Die Salze in den Versuchen wurden vor dem Gebrauch gut ausgetrocknet.

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in der Capillare noch mehrere Tage lebendig.[1])

Wie gross die Zahl der in dieser Weise in die Capillare angelockten Schwärmer sein konnte, kann man ungefähr ausrechnen. Da die durchschnittliche Grösse unseres Organismus ca. 10 μ uud 5 μ, und der innere Durchmesser des hier benutzten Capillarröhrchen 0,05 mm betrug so musste die wirkliche Zahl der im obigen Versuche eingedrungenen Individuen nicht weniger als 2 Millionen sein.

Die Bewegungsschnelligkeit der ganzen Zone der Schwärmer im Capillarinnere warn hier nur langsam, etwa 1,5 mm pro Stunde. Im Vergleiche dazu ist das Vorrücken der im normalen Zustande befindlichen Individuen aber sehr geschwind; es beträgt ca. 80 μ pro Secunde, also 288 mm pro Stunde.

Die starke Ansammlung, welche die Capillarmündung oft zum Verstopfen bringt, ist wieder von Bedeutung, da sie dem im Inneren enthaltenen Reizstoff eine schnelle Mischung mit der Aussenflüssigkeit erschwert und ihn eine Zeit lang auf den eingedrungenen Schwärmer seinen Reizeffekt ausüben lässt.

0,5% erzielte ähnliche jedoch nicht so starke Ansammlung wie bei 0,3%.

1% wirkte zuerst repulsiv, veranlasste aber nachher eine schwache Ansammlung um einer Entfernung der Capil-

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1) In einem derartigen Praeparate, welches am 24. October hergestellt wurde, waren die Schwärmer bis zum 27. November lebendig. Engelmann beobachtete sogar, dass „anscheinend völlig schwefelfreies *Bact. photometricum*, wenn es in H_2S-freien Wasser, in grosser Zahl in Glasröhren oder auf dem Objectträger unter dem Deckglas aufbewahrt ward, sich noch nach Wochen sehr lebhaft bewegt und auch sich vermehrt." (Die Purpurbacterien und ihre Bhezieungen zum Lichte. Bot. Ztg. 1888. p. 719).

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larmündung.

Ammoniumnitrat.

0,3%. Eine Ansammlung an der Capillarmündung.

Calciumnitrat.

0,3%. Eine schwache Ansammlung an der Capillarmündung.

Ammoniumtartrat.

0,5%. Eine momentane, reichliche Ansammlung um die Capillarmündung. Diese ordnete sich nach einigen Minuten zu einer kreisrunden Fläche, deren Durchmesser das Mehrfache des Capillardurchmessers betrug und die makroskopisch als rother Punkt erkennbar war. Die Schwärmer begannen dann langsam ins Capillarinnere vorzurücken und waren nach einer Stunde bis zu mässiger Tiefe vorgeschritten, jedoch blieb noch eine grössere Menge ausserhalb, nahe der Mündung. Wahrscheinlich wirkte hier eine 0,5% Lösung zu stark, um ein sofortiges Eindringen zu gestatten, welches erst resultirte, wenn die innere Lösung sich durch Diffusion langsam verdünnte. Ich bewahrte ein solches Capillarpraeparat, welches lebendige Zellen enthielt, in einer feuchten Kammer vom 13. November bis 16. December auf. Am letzt genannten Tage, als dasselbe Praeparat zur Untersuchung kam, war die rothe Färbung der bewegungslosen Zellhaufes in der Capillare noch nicht verschwunden und die Schwefeleinschlüsse in den Zellen noch immer z. Th. vorhanden. Nach 5 Minuten langem Einfluss des von einer Lampe reflektirten Lichtes aber wurden die Zellen, eine nach der anderen, bewegungsfähig und

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schwammen nach dem hinteren Theil des Capillarraums hin, während diejenigen Individuen, die in der Aussenflüssigkeit verblieben, verblassten und nicht mehr lebendig wurden.

Seignettesalz (Kaliumnatriumtartrat.)

0,3%. Eine starke Ansammlung an der Capillarmündung und eventuelles Eindringen ins Innere.

Ammoniumphosphat.

0,3%. Eine momentane, starke Ansammlung an der Mündung und darauffolgendes Eindringen.

Monokaliumphosphat (neutralisirt durch Natriumcarbonat.)

0,3%. Eine starke Ansammlung in dem oberen Theil des Capillarraums.

0,8%. Eine schwache Repulsion.

Natriumchlorid.

0,3%. Eine sehr schwache Anziehung.

0,8%. Eine Repulsion.

Ammoniumchlorid.

0,3%. Fast indifferent, oder sehr schwache Anziehung.

0,8%. Eine Repulsion.

Ammoniumsulfat.

0,3%. Eine Anziehung.

Magnesiumsulfat.

0,3%. Fast indifferent.

Natriumcarbonat.

0,3%. Eine Repulsion.

Natriumbicarbonat.

0,3%. Eine schwache Repulsion.

Kaliumchlorat.

0,3%. Eine Repulsion.

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Alaun.

0,3%. Eine Repulsion.

Ammoniak (sehr starke Lösung).

0,5%. Eine Repulsion. Die Repulsionssphaere hatte einen Durchmesser ca. 5 mal grösser als der Capillardurchmesser.

Rohrzucker.

0,5%. Eine schwache Ansammlung um die Capillarmündung.

Traubenzucker.

0,5%. Eine schwache Anziehung.

Milchzucker.

0,5%. Eine schwache Anziehung.

Asparagin.

0,5%. Eine schwache Anziehung.

Glycerin.

0,5%. Fast indifferent.

Pepton.

0,5%. Eine Ansammlung an der Mündung.

Fleischextrakt.

0,5%. Eine ziemlich starke Ansammluug an der Mündung.

Aepfelsäure.

0,5%. Eine momentane, starke Repulsion. Die dabei gebildete Repulsionssphaere hatte einen scharfen Umriss, mit einem Durchmesser ca. 43 mal grösser als derjenige von der Capillare (Fig. 22.) Diejenigen Individuen, welche in die Wirkungszone der Säure zufällig gekommen waren, kehrten bald nach Aussen zurück. Die Erscheinung dauerte mehrere Stunden lang, mi immer undeutlicher werdender Repulsionssphaere.

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Wie die oben beschriebenen Versuchsresultate hinreichend zeigen, ist unser *Chromatium Weissii* sehr deutlich mit der chemotaktischen Reizbarkeit begabt. Zu den besten Reizstoffen soweit unsere Versuche reichen, gehören neben dem Schwefelwasserstoff, Tartrate, Phosphate, Nitrate (hauptsächlich Kaliumnitrat), welche aber nur in schwächeren Concentrationen Anziehungen erzielen können, da sie bei geringer Concentrationssteigerung gleich Abstossungen veranlassen. Weder Fleischextrakt, Pepton, noch Zuckerarten, die sich in anderen Fällen[1]) als vorzügliche Reizstoffe erwiesen, kommen hier zu voller Geltung. Die Repulsionsmittel, die ich auffand, wirkten entweder nur durch die Concentration oder durch ihre specifischen Eigenschaften. Bedeutungsvoll ist aber ihre Reizbarkeit gegen H_2S, welche in Hinsicht der speciellen Lebensweise unserer Organismen eine wichtige Rolle spielt.

Ich hatte keine Gelegenheit auch andere freibewegliche, rothe Schwefelbacterien auf dieselben Erscheinungen zu prüfen, doch hege ich keinen Zweifel, dass diese Eigenschaft auch bei ihnen vorkommt.

Dass unser rother Organismus auch nach Art des Kontaktreizes reagirt, kann nicht geleugnet werden, da ich sehr oft ein Herumtummeln von Individuen um irgend einen festen Gegenstand wie Gyps-oder Schwefelkryställchen, oder dem Detritus organischer Körper beobachten konnte. Diese Erscheinung konnte ich durch das Zubringen frisch gefällter Gypskryställchen oder gereinigter Sandpartikeln öfters hervorrufen; es kann hier nicht eine chemische Reizung der angewandten Stoffe vorliegen, sondern ist, wie bei manchen Infusorien[2]) bekannt, an eine Art von Berührungsreiz zu denken.

Die Neigung unserer Bacterien, sich häufig anzusetzen und an

1) Vergl. Pfeffer, Ueber chemotaktische Bewegungen von Bacterien, Flagellaten und Volvocineen, (Unters. a. d. botan. Instit. z. Tübingen Bd. II. Heft III. 1888. p. 611.) und Miyoshi, Ueber Chemotropismusder Pilze, (Bot. Ztg. 1894. Heft I. p. 13.)

2) Vergl. Miyoshi, Physiologische Studien über die Ciliaten, (Botanical Magazine, Vol. X. No. 112. Tokio, 1896. p. 48.)

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den Flächen vom Objectträger und Deckgläschen anzuhaften, beruht wohl auf derselben Ursache, eine Erscheinung, die man bei *Paramœcium*, *Colpidium* und andere Ciliaten gewöhnlich wahrnimmt. Zuweilen sah ich nur eine geringe Anzahl von Individuen eine Zeit lang um ein Gypskryställchen sich herumtummeln, in andern Fällen aber kamen immer neue Individuen von der Umgebung herbei und so wuchs das ursprüngliche, kleine Klümpchen um ein Centrum zu einem grösseren Haufen an, welcher aber nach einiger Zeit sich wieder zum Schwärmen auflöste. Wie massgebend diese mechanische Reizung auch sein mag, um die Schwärmer zur Haufenbildung zu führen, darf man doch auch nicht vergessen, dass chemische und andere Reize solche Zellhaufen ebenfalls zu Stande bringen können.[1])

Zusammenfassung einiger Resultate.

1) Die Schwefelrasen von Bad Yumoto (Nikko) und Bad Schibu entstehen durch die Ablagerung des Schwefels auf die im H_2S-haltigen Thermalwasser (von ca. 51° bis 70°C) vorkommende, schleimige Bacteriengallerte. Im schnellfliessenden Strome fällt der Schwefel in amorphen Körnchen oder unvollkommenen Kryställchen aus; dann sehen die Rasen mehr weiss als gelb aus. Im langsam strömenden Wasser setzt sich das Element in grösseren Krystallen, meistens rhombischen Octaëdern ab, uud verleiht den Rasen eine mehr gelbe Farbe.

2) In den Gallertmassen ist eine unzählige Menge von sensenförmigen Bacterienzellen stets zu finden. Andersartige Zellformen treten gewöhnlich zusammen mit den ersteren in grösserer oder kleinerer Anzahl auf.

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1) In allerneuester Zeit beschrieb Jegunow das Zustandekommen eigenthümlicher Schwefelbacterien-Gesellschaften. (Bakt. Centralblatt. 2te Abth. 1896. Bd. II. No. 1. 14. 15.)

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3) In Yumoto kommen 4 farblose und 5 rothe Schwefelbacterien vor.

4) Das rothe Schwefelbacterium, *Chromatium Weissii* ist chemotaktisch reizbar: es wird durch verdünnte Lösungen von H_2S, Ammoniumtartrat, Kaliumnitrat, Ammoniumphosphat u. A. stark angelockt, dagegen durch höhere Concentrationen dieser Stoffe, sowie durch die specifische Eigenschaften gewisser anderen Stoffe abgestossen.

5) *Chromatium Weissii* ist durch Berührung reizbar, was oft zu einer dichten Haufenbildung führt.

Anhang.

Folgende Arten von Schwefelbacterien traten in einem Kulturbassin auf, welches einige Rhizomenstücke von *Brasenia peltata* aus dem Teiche im botanischen Garten der Universität nebst einen Zusatz von Gyps enthielt. Der Bassin stand in einem dunklen Raum des botanischen Institutes, ca. $^3/_4$ Jahre lang.

Beggiatoa alba (Vauch.) Trev. Als grauweisser Ueberzug auf den vermoderten Rhizomenresten.

Beggiatoa minima Winog. [Beitr. p. 25. Taf. I. fig. 3] mit der vorigen Art zusammen.

Thiothrix nivea (Rabenh.) Winog. Erschien als flockige, graue Büschel, welche in einer grossen Anzahl unmittelbar unter dem Wasserspiegel hiengen (Fig. 23). Die Büschelmasse war nur ca. 5 mm lang und adhärirte mit ihrem oberen, farblosen gallertartigen Theile an dünnen, aus Bacterienzoogloea und Kalkkrystallen bestehenden Häutchen. Der untere Theil der Büschel war dichter und dunkler wegen der Verwachsung der Fäden und der Ansiedelung einer anderen, der nächstfolgenden Schwefelbacterienart. Die Büschel-

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bildung kam auch an den inneren Bassinwänden zn Stande, jedoch war sie nur auf die oberste Wasserschichte beschränkt.

Thiosphaerion violaceum nov. Gen. et Sp. (Fig. 24). Zellen sphaero ellipsoidisch, ca. 2.5 μ und 1.8 μ gross, violett gefärbt, mit winzigen Schwefeleinschlüssen, durch eine gallertartige Substanz zu soliden, rundlichen Familien verbunden. Schwärmfähig. An den Fäden von *Thiothrix nivea*.

Thiosphaera gelatinosa nov. Gen. et Sp. (Fig. 25). Zellen sphaero-ellipsoidisch, 7 μ und 5 μ gross, leicht violett gefärbt, mit einer farblosen Gallertsubstanz zu einer Familienmasse locker verbunden. Schwefeleinschlüsse ziemlich zahlreich. Schwärmfähig. Auf dem Schlammboden.

Thioderma rubrum nov. Sp. (Fig. 26). Zellen ellipsoidisch, 4 μ und 2 μ gross, leicht roth gefärbt, zu einer leicht pfirsichschblüthrothen, abnehmbaren Haut verbunden. Schwärmfähig. Auf dem Schlammboden.

Im September 1896.

Tokio, Botanisches Institut
der Kaiserl. Universität.

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Figuren-Erklärung.

Die Figuren 1, 24, 25 sind von Herrn S. Hirase, Assistent an dem botanischen Institute, angefertigt worden. Alle übrige Zeichnungen sind von mir.

Fig. 1. Ein Schwefelrasenstük aus einem schnell fliessenden Thermalwasser in Yumoto, bei Nikko. In einem Becherglas flottierend. Nat. Gr.

Fig. 2. Ein Bruchstück eines vom Schwefel noch nicht ganz umgekleideten Gallertfadens mit sensenförmigen Bacterien. Der Schwefel krystallisirt in rhombischen Octäedern. Vergr. 420.

Fig. 3. Ein ähnliches Stück mit amorpher Schwefelablagerung. Vergr. 240.

Fig. 4. Ein Spitzentheil eines Gallertfadens mit spärlichen Schwefelkörnchen. Vergr. 240.

Fig. 5. Ein Bruchstück eines Gallertfadens eines im langsamen Strome vegetirenden Rasens. Der Schwefel setzt sich in Form grösserer Krystalle ab. Vergr. 240.

Fig. 6. Ein Bruchstück eines dünnen Fadens aus einer schnellen Strömung. Die Schwefelablagerung fand meistens in Form amorpher Körnchen selbst auf den dünnen Bacterienzellen statt. Vergr. 1000.

Fig. 7. Einzelne Bacterienzellen. Die Zellen führen auf den Zellwänden aufgelagerte Schwefelkörnchen, die oft wie im Zellinneren zu liegen scheinen. Vergr. 1000.

Fig. 8, a. b, c. Bacterienzellen in verschiedenen Grössen. Nur an zwei Individuen sind die Geiseln gezeichnet. Vergr. 1000.

Fig. 9. Ein Bruchstück des Schwefelrasens aus einen Thermalflüsschen im Bad Idsusan. Der mittlere, schwarze Theil besteht aus Schwefelkörnchen und Schwefelkryställchen, die randständigen Pünktchen stellen die aus der Gallerte hervortretende Bacterienmasse dar. Vergr. 420.

Fig. 10. Ein Spitzentheil eines ähnlichen Rasens. Die Schwefelkryställchen in Prismen und Octaëdern treten deutlich hervor. Vergr. 420.

Fig. 11, a. Ein ähnlicher Spitzentheil mit 2 langen *Leptothrix*ähnlichen Fäden. *b.* Einzelne Bacterienzellen. Vergr. 420.

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Fig. 12. Einzelne Bacterienzellen noch stärker vergrössert. Vergr. 1000.

Fig. 13. Thiothrix nivea, var. verticillata. Auf einem todten Algenfaden. Jüngere, kürzere Fäden zusammen mit älteren, längeren Fäden wirbelförmige, falsche Verästelung bildend. Vergr. 420.

Fig. 14. Thiothrix tenuis. Auf zwei mit Schwefelkryställchen z. Th. bedeckten Fäden von *Thiothrix nivea*. Vergr. 420.

Fig. 15. Chromatium Weissii. Vergr. 1000.

Fig. 16. Chromatium minus. Vergr. 1000.

Fig. 17. Chromatium vinosum. Vergr. 1000.

Fig. 18. Chromatium minutissimum. Vergr. 1000.

Fig. 19. Thioderma roseum. Vergr. 1000.

Fig. 20. Eine starke Ansammlung der Schwärmer von *Chromatium Weissii* um die Capillarmündung und auch im ersten Theile des Capillarraumes. Die Capillare enthielt 0.3% Ammoniumnitrat. Gezeichnet unmittelbar nach dem Einschieben der Capillare. Eine grosse Anzahl von frei schwimmenden Zellen befanden sich noch in der Aussenflüssigkeit. Die Querlinie stellt einen Rand vom Deckgläschen dar. Vergr. 85.

Fig. 21. Dasselbe Praeparat 8 Stunden nach dem Eintreten in die Capillare, (die Stellung der Capillare etwas geändert.) Die Schwärmermasse rückte immer weiter vorwärts. Vergr. 85.

Fig. 22. Eine durch 0,5% Aepfelsäure auf *Chromatium Weissii* erzielte Repulsionssphäre. Vergr. 50. Gezeichnet unmittelbar nach dem Einschieben der Capillare.

Fig. 23. Eine profile Darstellung der unter dem Wasserspiegel hängenden Büschel von *Thiothrix nivea*. Die Büschel haften mit ihrem oberen, farblosen, gallertartigen Theile auf den flottierenden Bacterien- und der Kalkkryställchenhaut. Der untere Theil sieht wegen des geselligen Verwachsens der Fäden sowie wegen der Ansiedelung anderer Schwefelbacteriencolonien mehr dunkel aus. Nat. Gr.

Fig. 24. Thiosphaerion violaceum. a. Kugeliche Colonien auf den Fäden von *Thiothrix nivea*. Vergr. 240. *b.* Einzelne Zellen. Vergr. 420. *c.* 2 Fadenstücke von *Thiothrix nivea:* das eine mit dichten Schwefelkörnern, das andere schwefelfrei; die falsche Gliederung sichtbar. Vergr. 790.

Fig. 25. Thiosphaera gelatinosa. Die locker gebundenen Zellen sind in einer gelatinösen hyalinen Grundsubstanz eingebettet. Vergr. 420.

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Fig. 26. Thioderma rubrum. Ein Bruchstück aus einen Zellhaufen. Vergr. 420.

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Die Entwickelung der Gonophoren bei Physalia maxima.[1)]

von

Seitaro Goto, *Rigakushi, Rigakuhakushi.*

Professor der Biologie an der Ersten Hochschule in Tokyo.

Hierzu Taf. XV.

Folgende Beobachtungen wurden an einigen, von meinem verehrten Lehrer, Herrn Prof. Dr. Brooks, vor einigen Jahren gesammelten und conservirten Materialien, welche er gütigst mir überlassen hat, ausgeführt. Ich habe sie mit besonderer Rücksicht auf die Frage nach der Natur des sogenannten weiblichen Gonophors (Haeckel) untersucht. Auch habe ich die Entwickelungsstadien des männlichen Gonophors studirt, und da es, meines Erachtens, eine bisher nicht beobachtete Besonderheit darbietet, und da Weismann in seiner Monographie über die Entstehung der Sexualzellen bei den Hydromedusen die vorliegende Art hat unberührt lassen müssen, so scheint es mir, dass es nicht ohne Wert sein wird, meine Beobachtungen etwas ausführlich zu beschreiben.

Männliches Gonophor.—Das jüngste von mir beobachtete Entwickelungsstadium des männlichen Gonophors ist in Fig. 1 wiedergegeben. Es ist eine einfache, aus Ento- und Ectoderm bestehende zitzenförmige Knospe des Stiels. Die Bildung des Glockenkernes

1) Dieser Aufsatz wurde im vorletzten Sommer zu Baltimore in englischer Sprache verfasst und zur Zeit meiner Abreise dort hinterlassen, um in einer amerikanischen Zeitschrift veröffentlicht zu werden. Da er aber bisher noch nicht im Druck erschienen ist, so übergebe ich eine deutsche Uebersetzung desselben hiermit der Oeffentlichkeit.—October 1896.

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hat schon begonnen, aber man findet keine Höhle im Entoderm der Knospe. Dieser Mangel einer vom Stiel aus in die Knospe hineintretenden Höhle mag das allererste Anfangsstadium bei der Entwickelung des Gonophors darstellen, oder aber, was mir wahrscheinlicher erscheint, erst sekundär durch eine äusserst, rege, zur Bildung der Keimzellen führende Zellenwucherung im betreffenden Entodermabschnitt hervorgebracht worden sein. Ich neige der letzteren Ansicht zu, weil ich in mehreren anderen Schnitten fast desselben Stadiums das Darmdivertikel beobachten konnte. Wie in Fig. 1 ersichtlich, ist die Form des Glockenkerns eine mehr oder weniger verlängerte, stellt aber zuweilen eine abgeflachte Kegelform dar, deren Zipfel nach dem Gipfel der Knospe gerichtet ist. Er enthält gewöhnlich eine mehr oder weniger geräumige Höhle, aber dieselbe besitzt nie eine so regelmässige Form, wie man sie bei vielen Hydromedusen beobachtet. Man muss jedoch bemerken, dass ihre Bildung meistenteils schon in dem ersten Entwickelungsstadium des Glockenkerns anfängt, und dass sie nachher zur Subumbrellarhöhle wird.

Im oben beschriebenen Stadium kann man die Kerne der Keimzellen deutlich beobachten, und ihre Einwanderung in den Glockenkern hat schon begonnen. Sie nehmen ihren Ursprung durch Teilung von den Entodermzellen der Knospe. Diese Angabe ist ausser Zweifel dadurch bewiesen, dass eine rege mitotische Teilung im betreffenden abschnitt stattfindet. Die in Teilung befindlichen Kerne liegen meistenteils sehr nahe an der freien Fläche des Entoderms der Knospe, wie man aus der aus einem späteren Stadium entnommenen Fig. 4 ersehen kann. Die Keimkerne sind durch ihre Kugelform und ihr bläschenartiges Aussehen charakterisirt; ihr Chromatin kommt in Form weniger, grober, meist an die Kernmembran sich anschmiegender Klümpchen vor. Sie sehen also in den Schnittbildern klarer aus, als die Kerne der gewöhnlichen Ento-

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dermzellen, bei welchen das Chromatin gleichmässiger verteilt ist, und welche ein dunkleres Aussehen haben. Es unterliegt kaum einem Zweifel, dass diese Eigentümlichkeit der Keimkerne teilweise davon herrührt, dass sie einen sehr regen Teilungsvorgang durchgehen, und die bei der Teilung in die bestimmte Zahl der Chromosomen gesammelte chromatische Substanz nicht Zeit genug hat, wieder im Kerne als feine Körnchen verteilt zu werden.

Zur Rechtfertigung dieser Angabe führe ich die Beobachtung an, dass auch bei den interstitiellen Zellen und im Subumbrellarektoderm des Gonophors, die Tochterkerne nach der Teilung mehr oder weniger eine Kugelform annehmen, und dass ihre chromatische Substanz sich dabei in wenigen grösseren Klümpchen ansammelt. Doch muss zugegeben werden, dass die oben angeführte Eigentümlichkeit stets von den Keimkernen festgehalten wird ; so habe ich beobachtet, dass selbst in den beinabe reifen männlichen Gonophoren, in welchen die Umbildung in Spermatozoen schon begonnen hat, die Keimkerne noch durch ihr bläschenartiges Aussehen ausgezeichnet sind.

In der obigen Beschreibung habe ich das Wort „ Keimkerne " statt „ Keimzellen " gebraucht. Dies geschieht deshalb, weil in dem betreffenden Abschnitt keine Zellengrenze zu beobachten ist, indem die Kerne in einer continuirlichen Protoplasmamasse eingelagert sind.

In Fig. 1 sieht man zwei Keimkerne schon in den Glockenkern eingewandert, und ein dritter befindet sich auf seinem Wege durch die Stützlamelle. Ich habe sämtliche Stadien dieser Einwanderung, von demjenigen, in welchem man einen Kern die Stützlamelle vor sich hervorstülpen sieht, bis zu demjenigen, in welchem ein Kern dieselbe eben zurückgelegt hat, beobachten können.

Nun möchte ich einer schon oben erwähnten Eigentümlichkeit in der Bildungsweise des Glockenkerns gedenken, einer Besonderheit, welche, wie mir erscheint, allgemeiner vorzukommen pflegt. Der

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Glockenkern nämlich verdankt bei der vorliegenden Art seinen Ursprung der Einwanderung der interstitiellen Zellen des Ektoderms. Dieselben lassen die Stützlamelle vor sich gegen den Entoderm einstülpen und bilden den Glockenkern, dessen Form schon oben beschrieben worden ist. Meines Wissens hat Niemand das Augenmerk auf diese Besonderheit gerichtet, aber eine Durchmusterung zahlreicher dazu geeigneter Schnitten hat mir die Richtigkeit meiner Beobachtung aussser Zweifel gestellt. An einigen Schnitten habe ich sogar das Einwandern der interstitiellen Kerne fast sehen können, jeder Kern mit einem Protoplasmastrom um sich herziehend. Um diese rege Einwanderung zu gestatten, müssen die interstitiellen Zellen entweder in genügender Zahl von aussen her in die Gonophorknospe einwandern, oder aber sie müssen in der Knospe selbst sich wiederholt teilen. Aus meiner Beobachtung, dass unter den eingewanderten Zellen nie die karyokinetische Figur, und nur äusserst selten ein auf kürzlich vollzogene Teilung andeutendes Kernbild zu sehen war, glaube ich schliessen zu dürfen, dass die den Glockenkern bildenden Zellen in der ersteren Weise ihren Ursprung nehmen. Der in Fig. 1 an der linken Seite des Gipfels der linken Entodermlamelle eingezeichnete Kern ist der einzige, den ich beobachtet habe, der im Wiedererbauen des ruhenden Kernes von einer Teilung begriffen zu sein scheint. Dass der Glockenkern den eingewanderten interstitiellen Zellen seinen Ursprung verdankt, erscheint mir um so wahrscheinlicher, da keine Teilung im definitiven Ektoderm stattfindet, und da der Ektoderm oberhalb der Einwanderungshöhle völlig unberührt bleibt.

Betreffend die Ursache des Einstülpens des Entoderms, um den der Bildung des Glockenkerns notwendigen Raum zu geben, so erscheint mir dieselbe nicht eine bloss mechanische zu sein, sondern ich glaube dass sie wenigstens teilweise in einem von dem bloss

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Mechanischen unabhängigen, im Entoderm einwohnenden Wachstumsgesetz zu suchen ist. Diese Ansicht wird dadurch wesentlich gestützt, dass, wie schon oben erwähnt, in fast allen Schnitten des ersten Entwickelungsstadiums des männlichen Gonophors ein Raum, sei er gross oder klein, schon vorhandan ist, und dass die interstitiellen Zellen erst nachträglich in diesen Raum einzuwandern scheinen.

In Fig. 1 sieht man auch die Anlage der Radialkanäle (*can. rad.*), welche durch die auf bestimmte Gebiete eingeschränkte Verwesung der Zellen der Entodermlamelle zur Ausbildung gelangen. In Fig. 1 giebt es zwei Räume, einen kleineren und einen grösseren. Im grösseren Raum sieht man eine körnige Masse, und im kleineren ein intensiv sich färbendes Körperchen. Durch eine Vergleichung mehrerer Schnitten bin ich zu dem Schlusse gelangt, dass dieselben Ueberbleibsel der am Orte der Verwesung anheimgefallenen Zellen darstellen. Ich habe sämtliche Stadien in diesem Verwesungsprocess beobachten können. Im Anfangsstadium wird die chromatische Kernsubstanz zu einer die Mitte des bläschenartig gewordenen Raums einnehmenden, einzigen Masse verschmolzen, dann verschwindet allmählich die Kernmembran, und so erhält man eine solche Höhle, wie man sie in Fig. 1 sieht. Den Uebergangsprocess von diesem Stadium bis zu der grossern Höhle in derselben Figur dürfte man in der Weise sich vorstellen, dass dieses Körperchen sich zu einer körnigen Substanz auflöst, und dass gleichzeitig der Raum sich vergrössert. Die körnige Masse verschwindet zuletzt gänzlich.

Die Bildung der Entodermlamelle hat schon in Fig. 1 begonnen, und sie ist in Fig. 2 etwas weiter fortgeschritten. In diesem Stadium sind die zwei Radialkanäle völlig ausgebildet und erstrecken sich bis zum Gipfel der Entodermlamelle. Querschnitte der Gonophorknospe zeigen, dass die Entodermlamelle in zwei entgegengesetzten Quadranten eine grössere Dicke besitzt, als in den übrigen zwei, wo

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dieselbe mit Ausnahme des dem Gipfel angrenzenden Abschnitts zu einer sehr dünnen Schicht geworden ist, und dass die Radialkanäle sich in den verdickten Quadranten befinden (Fig. 3). Obgleich durch die örtliche Verwesung der Zellen gebildet, besitzen die Radialkanäle in diesem Stadium eine deutliche dünne Membran, welche entweder von dem umliegenden Protoplasma ausgeschieden worden ist, oder doch wohl nur eine Verdickung desselben darstellt.

Fig. 2 stellt einen im rechten Winkel zu der die zwei Radialkanäle einnehmenden Ebene geführten Schnitt dar. Die Einwanderung der interstitiellen Zellen setzt sich fort, und einige der eingewanderten scheinen der Verwesung anheimzufallen, und so zur Bildung der inzwischen zu einer ansehnlicheren Grösse herangewachsenen Subumbrellarhöhle beizutragen. Die in der Subumbrellarhöhle oft vorkommende körnige Masse kann man durch sämtliche Stadien der Verwesung in die eingewanderten Kerne verfolgen. Die Auswanderung der Keimkerne findet noch in diesem Stadium statt; sie bilden an der Basis der Subumbrellarhöhle eine halbkugelförmige Masse, in welcher keine Zellgrenze zu erkennen ist. Die ganze Subumbrellarhöhle ist mit einer einschichtigen, aus den eingewanderten Zellen bestehenden Zellenschicht ausgekleidet, deren Kerne sehr ungleichmässig verteilt sind. Ausser diesen zu einer Schicht verbundenen Kernen giebt es noch andere, welche zwischen den Keimkernen zerstreut sind. Man erkennt unterhalb der Keimkernmasse eine leichte Ausbuchtung der Darmhöhle; dies ergiebt sich als die Anlage des Spadix.

In Fig. 2 sieht man, dass die Keimkerne nicht am Gipfel des künftigen Spadix, sondern nur an den seitlichen Teilen ihren Ursprung nehmen. Auf diesen Umstand hat man mit Nachdruck verweisen; aber eine Durchmusterung zahlreicher Schnitte hat mich gelehrt, dass dies in keiner Weise immer der Fall ist, sondern dass

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auch die Kerne der Entodermkuppe wiederholt sich teilen, und dass die daraus gebildeten Keimkerne nach der Stützlamelle activ auswandern.

Das weitere Wachstum und die weitere Entwickelung des männlichen Gonophors vollziehen sich durch Veränderungen in den Anlagen, welche man in den im Obigen beschriebenen Stadien gesehen hat. Der Spadix strebt nach seiner definitiven Ausbildung, und damit entwickelt sich an seiner Basis ein enges, nach aussen gerichtetes Darmdivertikel, welches in die Radialkanäle sich fortsetzt und zugleich als Wegweiser zu der mit der Bildung der Entodermlamelle verknüpften Faltenbildung dient. Mit der Ausbildung des Spadix kann man von dem Manubrium reden. Die Auswanderung der Keimkerne scheint zum Stillstand gekommen zu sein, und die in mehreren Schnitten zu beobachtenden Teilungen führen aller Wahrscheinlichkeit nach nur zur Bildung der zu dem später eintretenden ansehnlichen Wachstum der Knospe nötigen Entodermzellen. Die von der Entodermlamelle frei gelassene Oeffnung am Gipfel der Knospe ist zu dieser Zeit durch das Zusammentreffen und die Verwachsung des Randes der Stützlamelle geschlossen, und damit kommt die Einwanderung der interstitiellen Zellen des Ektoderms zum völligen Abschluss. Die zum Schliessen der obenerwähnten Oeffnung sich anschickende Stützlamelle sieht vor demselben ganz wie die des Velums aus.

Die Keimkerme teilen sich mehrere Male im Manubrium, der Spadix verlängert sich, und die ganze Knospe wächst an, bis sie ein solches Stadium erreicht, wie es Fig. 5 wiedergiebt. Diese Figur stellt einen Axialschnitt durch ein beinahe reifes Gonophor dar. In diesem Stadium sind die Radialkanäle völlig zum Verschwinden gekommen; die Entodermlamelle aber sieht man noch, obgleich sie an einigen Stellen zu einer äusserst dünnen Schicht geworden ist.

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Ihr freies Ende bleibt dauernd getrennt, und der Zwischenraum ist nur von einer dünnen Stützlamellemembran ausgespannt. Im Gegensatz zu den Keimkernen, welche durch wiederholte Teilungen sich vermehren, zeigen die eingewanderten Kerne kein Zeichen der Vermehrung, und folglich trifft man sie nur hie und da in der die Subumbrellarfläche auskleidenden Protoplasmaschicht. Das Manubrium besitzt keine deutliche äussere Auskleidung, und die Kerne findet man äusserst spärlich unter den Keimkernen zerstreut.

Das Ektoderm der Knospe zeigt an deren Gipfel eine Verdickung; ihre Zellen färben sich meist sehr intensiv, und man ist geneigt, in ihnen einzellige Drüsen zu erblicken. Diese Verdickung ist schon von Weismann bei einigen anderen Arten beobachtet worden, und ist nach seiner Meinung die Stelle, wo die Knospe später zum Ausbruch kommt.

Die Zellen des Spadix sind nicht nur sehr gross geworden, sondern sie enthalten auch Vacuolen und sehen jetzt den Entodermzellen des Stieles ähnlicher, als in ihren früheren Stadien, wo die Zellleibessubstanz sehr feinkörnig war und sich mit dem Farbstoff intensiv färbte.

In mehreren Keimkernen sieht man die chromatische Substanz halbmondförmig an einer Seite des Kernes dicht an der Kernmembran angesammelt. Meines Erachtens ist dies der erste Schritt zur Ausbildung der Spermatozoen.

Das Ektoderm der Knospe ist in diesem Stadium der interstitiellen Zellen völlig leer.

Sogenanntes weibliches Gonophor.—Dieses beschreibt Haeckel in seiner Challenger- Monographie folgendermassen: „Their umbrella is well developed, campanulate or pyriform, with four radial canals and a ring-canal, and a broad velum around the ostium. The manubrium, in the wall of which the eggs ripen, seems to be developed usually after

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the detachment. It is not visible in the sessile gynophore, or forms only a small tubercle in the center of the subumbrella. The apex of the gynophore is attached to the branch of the stem by a large conical pedicle."

Das jüngste Stadium des weiblichen Gonophors, das ich zur Beobachtung erhalten konnte, war schon viel älter, als dasjenige des männlichen Gonophors; es ist in Fig. 6 wiedergegeben, welche nur den distalen Teil eines Längsschnittes zeigt. Der Glockenkern ist schon ganz hohl; es unterliegt aber kaum einem Zweifel, dass er in derselben Weise wie derjenige des männlichen Gonophors gebildet worden ist. Die in der Einwanderung begriffenen Zellen habe ich in mehreren Schnitten beobachten können, und kann dies auch in Fig. 6 gesehen werden. Man sieht nämlich im Ektoderm der distalen Hälfte der Knospe mehrere interstitielle Zellen; einige derselben befinden sich an dem künftigen Ostium, welches in diesem Exemplar von einer Zellenmasse geschlossen ist. An dieser Zellenmasse erkennt man, dass sie aus den vom Ektoderm her eingewanderten interstitiellen Zellen gebildet ist. Ihr Aussehen und ihre Form sind denjenigen der interstitiellen Zellen des Ektoderms völlig gleich, nur haben sie etwas mehr Protoplasma; und einige derselben haben sich regelmässig angeordnet, um ein Epithel zu bilden. In Fig. 6 sieht man auch, dass die eingewanderten, im künftigen Ostium befindlichen Zellen durch ihr Anhäufen eine leichte Hervorwölbung des Ektoderms verursacht haben. Dieser Anhäufungsprocess setzt sich fort, und wenn alle interstitiellen Zellen ihre Einwanderung vollendet haben, so bleibt an der Stelle der angehäuften Zellenmasse eine mit der Umbrellarhöhle frei communicirende Höhle übrig, welche nach aussen nur durch eine äusserst dünne Ektodermschicht begrenzt ist. Diese Schicht bricht zuletzt durch, und führt gerade zur Bildung des Ostiums.

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In diesem Stadium ist die Anlage des Velums schon vorhanden; sie ist ein aus der Stützlamelle bestehendes Diaphragma, welches vom freien Ende der Entodermlamelle frei hervorragt.

Wie in den männlichen Gonophoren, fallen viele der eingewanderten Zellen der Verwesung anheim, und die Subumbrellarhöhle enthält folglich in diesem Stadium eine etwas körnige Substanz und verwesende Kernkörperchen. Spuren der teilweise der Verwesung anheimgefallenen Kerne sieht man in Fig. 6 (zwei an jeder Seite).

Eine andere an den männlichen Gonophoren nicht deutlich zu beobachtende Tatsache tritt in den weiblichen Gonophoren klar zu Tage. Es ist die, dass viele der interstitiellen Zellen, statt in die Subumbrellarhöhle einzuwandern, Protoplasmafortsätze nach der Oberfläche wie auch nach der Stützlamelle schicken, und an der Bildung des definitiven Ektoderms teilnehmen, indem sie eine Form annehmen, welche derjenigen der definitiven Ektodermzellen ganz ähnlich ist. Man kann sämtliche Stadien in diesem Umwandlungsprocess beobachten, von den Zellen, welche noch tief im Ektoderm liegen, aber schon mit kurzen Protoplasmafortsätzen ausgestattet sind, bis zu denjenigen, in welchen die Fortsätze schon die Cuticula einerseits, und die Stützlamelle andererseits erreicht haben, deren Kerne jedoch noch tiefer gelagert sind, als diejenigen des definitiven Ektoderms. Ein derartiger Umwandlungsprocess lässt sich auch in den jungen Gonopalponen und den Siphonen beobachten, und ich glaube, dass derselbe in eingeschränkterer Masse nicht nur in den männlichen Gonophoren, sondern in allen sich entwickelnden Teilen des Tierstocks stattfindet.

In dem im Obigen beschriebenen Stadium sind die vier Radialkanäle, sowie der Ringkanal völlig ausgebildet. Die Ersteren besitzen eine einschichtige zellige Wand; in den Entodermlamellen aber liegen die Zellen unregelmässig geschichtet. Die Radialkanäle

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verursachen leichte Hervorwölbungen im Subumbrellarektoderm, so dass die Umbrellarhöhle in Querschnitten etwas kreuzförmig aussieht.

Der Stiel des weiblichen Gonophors ist verhältnissmässig lang ; die Stützlamelle ist allenthalben noch sehr dünn.

Im völlig ausgebildeten weiblichen Gonophor kann man zwei Abschnitte deutlich unterscheiden, den Umbrellarteil und den Stiel. Herr Prof. Dr. Brooks teilt mir mit, dass bei den lebenden Exemplaren dasselbe eine pulsirende Bewegung ausübt, und dass es einer bedeutenden Grössenveränderung fähig ist. Diese Angabe ist durch die histologischen Befunde gestützt : das ganze Gonophor, aber besonders der Stiel, ist stark muskulös und besitzt eine dicke, gallertartige Stützschicht. Das Ektoderm ist eine direkte Fortsetzung desjenigen des Gonostyls, aber er hat ein ganz anderes Aussehen. Im Gegensatz zu den Zellen des letzteren, welche meist niedrig sind und schwach sich färbende Kerne, sowie eine deutliche Cuticula besitzen, sind diejenigen des weiblichen Gonophors sehr hoch und besitzen intensiv sich färbende Kerne. Das Cytoplasma ist sehr feinkörnig, oder genauer gesagt, es besteht aus einem feinsten Reticulum (Fig. 12). Der äussere Rand jeder Zelle ist gewölbt, so dass der Umriss des Gonophorstiels mehr oder weniger wie in kleinen Abständen eingeschnitten aussieht. Der Mangel einer Cuticula ist auch auffallend. Dies bemerkt man mit Vorteil in einem solchen Schnitte, wie es Fig. 12 wiedergiebt, welche einen nahe dem Anheftungspunkt des Stiels geführten Querschnitt darstellt. In diesem Teil behalten die Zellen eines bestimmten Ektodermabschnitts die Beschaffenheit der Ektodermzellen des Gonostyls ; seine Zellen besitzen, wie an der oberen Seite der Fig. 12 gezeigt ist, schwach sich färbende Kerne und ein Cytoplasma ebenderselben Beschaffenheit, und sind mit einer deutlichen Cuticula versehen, ganz wie es im Gonostyl der Fall ist.

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Wie man aus Fig. 8, in welcher die Grenze des betreffenden Abschnitts mit einem Sternchen bezeichnet ist, ersieht, erstreckt sich dieser Abschnitt der Länge nach bis etwa $\frac{1}{3}$ der Länge des ganzen Gonophors, und scheint immer an der Abaxillarseite des Gonophorstiels zu liegen.

Die Muskelfibrillen des Ektoderms sind äusserst fein. In den Querschnitten des Stiels sehen sie wie kleine, sich der Stützlamelle dicht anschmiegende Pünktchen aus; sie sind aber sehr zahlreich. Selbst in Fig. 12 sind sie etwas grösser eingezeichnet, als sie in der Natur sind. In dem schon oben erwähnten Abschnitt, wo das Ektoderm durch eine andere Beschaffenheit ausgezeichnet ist, sind die Muskelfibrillen an den Fortsätzen der Stützlamelle angeordnet und rufen so die Bildung der sogenannten Muskellamellen hervor.

Die Stützlamelle ist von sehr wechselnder Dicke und enthält mehr oder weniger zahlreiche Fasern. In der distalen Hälfte des Gonophors wird sie von zahlreichen, ein körniges Protoplasma enthaltenden Kanälchen durchsetzt (Fig. 11). Diese Kanälchen kommen nicht nur in den weiblichen Gonophoren vor, sondern man begegnet ihnen auch zahlreich in allen Teilen des Tierstocks, wo die Stützlamelle eine bestimmte Dicke übertrifft. In den letztgenannten Teilen sind die Kanälchen nicht nur mit einem körnigen Protoplasma gefüllt, sondern sie enthalten auch Kerne; zuweilen enthält jedes Kanälchen nur einen einzigen Kern, zuweilen mehrere. Die Beschaffenheit des enthaltenen Protoplasmas, sowie der Kerne beweist, dass dieselben dem Entoderm angehören. In den Tentakeln tritt dies mit besonderer Klarheit zu Tage; hier sieht man, dass einige Entodermzellen sich unmittelbar in die die Stützlamelle durchsetzenden Kanäle fortsetzen. Zwar muss man zugeben, dass in den weiblichen Gonophoren kein Grund dazu liegt, das in den Kanälchen enthaltene Protoplasma von dem Entoderm oder von dem Ektoderm abzuleiten;

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aber die oben angeführte Tatsache dürfte uns berechtigen, das betreffende Protoplasma als dem Entoderm angehörend anzusehen.

Meines Erachtens dient dieser unmittelbare Zusammenhang der beiden Körperschichten zum Ernähren des Ektoderms, und ist wahrscheinlich dadurch herbeigeführt, dass die Stützlamelle eine bestimmte der Ernährung im Wege stehende Dicke erreicht hat. Von diesem Standpunkte aus dürfte man diesen Zusammenhang dem protoplasmatischen Zusammenhang der Pflanzenzellen vergleichen, da beide dem Zuführen der ernährenden Flüssigkeit, sowie vielleicht dem Leiten der Erregung dienen.

Die Entodermzellen des Gonophorstiels sind, wie die Ektodermzellen desselben, cylindrisch, aber ihr Cytoplasma ist etwas grobkörniger und färbt sich intensiver, als jene. Die Kerne sind meistenteils im basalen Teil gelagert.

Die entodermalen Muskelfibrillen sind bedeutend dicker, als die ektodermalen, und in den Längsschnitten des weiblichen Gonophors sieht man, dass die Zahl der Fibrillen beinahe derjenigen der Zellen entspricht ; jede Zelle enthält also eine einzige Fibrille in ihrer Basis. Die Fibrillen sind nicht der Stützlamelle dicht angeschmiegt, sondern stehen gewöhnlich in einer kleinen Entfernung von derselben. Die entodermalen Fibrillen des weiblichen Gonophors sind viel grösser, als die entsprechenden Fibrillen des Gonostyls oder selbst des Stammes.

Die Entodermachse des Gonophorstiels nimmt nicht dessen Mitte ein, sondern liegt bis ungefähr zum Ende des Stiels stets nach einer Seite, und zwar der abaxillaren, gedrängt, und erst dort zieht sie nach der Mitte zu, um sich mit der Entodermlamelle zu vereinigen.

Ich werde nun zur Beschreibung des Umbrellarabschnitts übergehen. In allen mir zur Beobachtung gekommenen Exemplaren ist dieser Abschnitt nur ein wenig grösser, als der Stielabschnitt.

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Die Radialkanäle, sowie der Ringkanal sind wohlausgebildet. Die Ersteren kommen in der Regel in der Vierzahl vor, aber einmal beobachtete ich ein mit fünf Radialkanälen versehenes weibliches Gonophor. Auch das Velum ist wohlausgebildet, und hängt lose von dem Ostium ab, ähnlich wie bei den meisten zeitlebens freischwimmenden Hydromedusen (Fig. 8 & 10). Das Manubrium erkennt man in Form einer an der Basis der Subumbrellarhöhle gelegenen Hervorwölbung des Ektoderms. Bei einigen Exemplaren konnte dasselbe schwer als solches erkannt werden, aber eine Vergleichung mehrerer Exemplare beweist zur Genüge, dass es immer vorkommt. Der Spadix ist noch nicht ausgebildet.

Das Ektoderm (ex- als auch subumbrellare) und die Entodermlamelle sind durch eine gallertartige Stützschicht von wechselnder Dicke voneinander getrennt. Diese Stützschicht schickt diaphragmenartige Fortsätze aus, welche entsprechende Falten des Ektoderms hervorrufen (Fig. 8 & 10).

Das Exumbrellarektoderm besitzt bis an das Velum dieselbe Beschaffenheit, wie dasjenige des Stiels; aber hier nimmt es ein anderes Aussehen an: Die Kerne sind nun von wechselnder Grösse, ihre Färbbarkeit ist ungleichmässig geworden, und das Protoplasma vacuolenreich; mit einem Worte, die Zellen sind denjenigen des gleich zu beschreibenden Subumbrellarektoderms sehr ähnlich. Die Längsmuskelfibrillen kommen auch im Exumbrellarektoderm vor.

Im Gegensatz zu den Zellen des Exumbrellarektoderms sind die Zellen des Subumbrellarektoderms sehr reich an Vacuolen, welche besonders im distalen Teil des Protoplasmas vorkommen. Die Kerne sind von wechselnder Grösse und ihre Färbbarkeit ist weniger gleichmässig, als bei dem exumbrellaren Ektoderm. Muskelfibrillen kommen im Subumbrellarektoderm nicht vor; statt ihrer habe

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ich zahlreiche, stark sich färbende Körner von starkem Lichtbrechungsvermögen, welche besonders um die oben erwähnten Fortsätze der Stützschicht in Haufen vorkommen, beobachtet. Die Aehnlichkeit dieser Körner mit den Querschnittsbildern der entodermalen Muskelfibrillen ist sehr auffallend; man kann jedoch durch Vergleichung der Quer- und Längsschnitte sich davon überzeugen, dass sie nicht Querschnittsbilder der Fibrillen darstellen. Ihre Bedeutung ist mir jedoch im Dunkel geblieben.

Die Entodermlamelle, wie auch die Wände der Radialkanäle bestehen aus in einer einzigen Schicht angeordneten, grossen, cubischen oder cylindrischen Zellen mit einem feinkörnigen Protoplasma, welches sich weniger stark färbt, als dasjenige des Exumbrellarektoderms (Fig. 9 & 10). Nach meiner Beobachtung mangelt dem Umbrellarabschnitt jegliche entodermale Muskel.

Allgemeine Bemerkungen.—Die im Vorhergehenden beschriebene Bildungsweise des Glockenkerns erscheint mir eine interessante Uebergangsform zwischen den gewöhnlichen Formen, bei welchen derselbe als eine solide Zellenmasse angelegt ist, und der von Weismann bei *Coryne pusilla* beobachteten.[1] Bei der ebengenannten Art wandern die jungen Ektodermzellen einzeln durch die Stützlamelle, ohne dieselbe vor sich einzustülpen, und erst nachträglich bilden sie den Glockenkern. Wir haben oben gesehen, dass wenigstens bei Physalia es die interstitiellen Zellen sind, welche vom Ektoderm her einwandern. Dieselben treiben die Stützlamelle jedoch vor sich und führen ohne Weiteres zur Bildung der Entodermlamelle und des Glockenkerns, wie es bei den meisten bisher untersuchten Formen der Fall ist. Ich habe auch Schnitte von *Bougainvillia* und *Pennaria* verfertigt, um wenn möglich eine ähnliche Bildungsweise des

1) Entstehung der Sexualzellen bei den Hydromedusen. S. 53 u.w.

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Glockenkerns zu beobachten ; aber teils aus Mangel an den richtigen Stadien, teils aus der Kleinheit der Elemente bei diesen Formen habe ich bisher keinen Erfolg darin gehabt.

In Bezug auf die Natur des sogenannten weiblichen Gonophors giebt es meines Wissens zwei Ansichten, nämlich diejenige von Haeckel, und diejenige von Brooks und Conklin.

Haeckel sieht das fragliche Gebilde als echtes weibliches Gonophor an und glaubt, dass der Umbrellarabschnitt von dem Tierstock hinabfällt und erst nachher als freischwimmende Qualle die Geschlechtsreife erreicht.[1] Dies glaubt er aus seiner Beobachtung einiger, nur die Stiele des fraglichen Gebildes besitzenden Exemplare von Physalia schliessen zu dürfen. Es scheint jedoch, dass er die Keimzellen nicht beobachtet hat.

Brooks und Conklin haben ihre Ansicht in ihrer Mitteilung über eine der Ordnung Auronectæ angehörende Siphonophore ausgesprochen.[2] In Bezug auf die vorliegende Frage sagen sie: „It seems proper here to mention the fact that an examination of the sexual organs of forty or fifty specimens of Physalia has shown that they were all males. The structures described by Haeckel as the female gonophores of Physalia are not gonophores, but swimmbells, as is shown by an examination of living expanded specimens."

Wie man aus dem Vorhergehenden ersieht, liefern meine Beobachtungen keine Spur der weiblichen Keimzellen ; dies kann jedoch gegen die Auffassung Haeckels nicht angeführt werden, da Weismann gezeigt hat, dass bei Velella und Porpita die Keimzellen erst nach der Trennung des weiblichen Gonophors vom Tierstock entwickelt werden.[3] Auf der anderen Seite spricht die muskulöse Beschaffenheit des Gebildes für die Brooks-Conklin'sche Auffassung.

1) Challenger- Report, S. 313.
2) Johns Hopkins Univ. Circulars. Vol. X, No. 88.
3) A. a. O. S. 212.

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Falls aber diese die richtige ist, so kennt man von dem Weibchen der Physalien nichts. Es würde vielleicht nicht ausser Platz sein, wenn ich erwähne, dass Bedot[1] durch eine neuere Beobachtung über eine junge Vellelalarve ein Zeichen ihrer Trennung von einem Muttertiere nachgewiesen hat, und dass danach die Lebensgeschichte der Siphonophoren wohl etwas verwickelter verläuft, als man sich vorzustellen gewohnt ist.

Es ist mir eine angenehme Pflicht am Schlusse dieser Mitteilung meinem verehrten Lehrer Herrn Prof. Dr. Brooks, meinen wärmsten Dank auszusprechen für die Freundlichkeit, mit welcher er mir das Material übergeben hat, sowie für seinen gedankenanregenden Rath.

1) Notes sur une larve de Velelle. Revue suisse de Zoologie et Ann. du Mus. d'hist. nat. de Genève, t. II, fasc. 4, 1894. S. 463.

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TAFEL XV.

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Tafel XV.

N. B. Die nach den arabischen Nummern befindliche wagerechte Linie (—) bedeutet „linear."

can. rad......Radialkanal.
can. cir......Ringkanal.
ect'd...... Ektoderm.
ect'd. exumbr......Exumbrellarektoderm.
ect'd. subumbr. ...Subumbrellarektoderm.
ent'd. ..... Entoderm.
ent'd lam...... ...Entodermlamelle.
gl'ck. k'n......Glockenkern.
nucl. germ......Keimkern.
sp'd'x. ..... Spadix.
vel'm. ..... Velum.

Männliches Gonophor.

1. Längsschnitt durch ein sehr junges Gonophor. × 190—.
2. Längsschnitt durch ein älteres Gonophor. × 190—.
3. Querschnitt durch ein Gonophor von demselben Stadium wie das Vorige. × 190—.
4. Längsschnitt durch ein Gonophor, bei welchem der Spadix sich anlegt. × 190—.
5. Längsschnitt durch ein beinahe reifes Gonophor. × 190—.

Sogenanntes weibliches Gonophor.

6. Längsschnitt durch den Umbrellarteil eines jungen Gonophors. × 190—.
7. Querschnitt durch den Umbrellarteil eines jungen Gonophors von demselben Stadium wie das Vorige. × 190—.
8. Längsschnitt durch ein älteres Gonophor. × 50—.
9. Ein Teil der Wandung des Umbrellarteils eines älteren Gonophors im Querschnitt. × 190—.
10. Dem Ostium angrenzender Teil der Wandung eines älteren Gonophors. × 190—.
11. Ein kleiner Teil des Längsschnittes durch ein älteres Gonophors. × 190—.
12. Querschnitt durch den Stiel eines älteren Gonophors. × 190—.

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Studies of Reproductive Elements.

III. Die Entwicklung der Pollenkörner von Allium fistulosum L., ein Beitrag zur Chromosomenreduktion im Pflanzenreiche.

von

Dr. C. Ishikawa.

Professor der Zoologie an der kaiserlichen Universität zu Tokyo.

(Tafeln XVI und XVII).

Angeregt durch die grosse Gedankenarbeit A. WEISMANN's (37) können wir jetzt, auf dem zoologischen Gebiete, als festgestellt annehmen, dass in dem Entwicklungscyclus der Genitalzellen eine Herabsetzung der Chromosomenzahl vorkommt, und zwar auf die Hälfte. Diese Reduction geschieht bei den letzten Theilungen der Ei- und Samenzellen, was allerdings noch von einzelnen Autoren bestritten wird. In vielen Fällen erkennt man aber in den Urgeschlechtszellen die Verminderung der Anzahl sehr deutlich, worauf durch zweimalige Spaltungen die Zahl sich wieder vermehrt, und dann sich in den Eizellen durch die Bildung der Richtungskörper wieder auf die Hälfte der ursprünglichen Zahl vermindert, ebenso auch in den Samenzellen bei den letzten Theilungen derselben.

Diese Verallgemeinerung wurde aber von botanischer Seite nicht anerkannt, obwohl ähnliche Phenomena von vielen Forschern beobachtet waren. So beobachteten GUIGNARD (10), STRASBURGER (33–36), FARMER (6), BELAJEFF (1), SARGANT (32) und andere die reducierte Zahl der Chromosomen auch in jungen Geschlechtszellen

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vieler Gewächse ; sie deuten aber diese in ganz anderer Weise als VOM RATH (25–29), HÄCKER (12–17), RÜCKERT (30,31) u. a. m. Während letztere Zoologen die reducierte Zahl nur für eine scheinbare halten, betrachten die Botaniker dieselben für eine thatsächliche ; ferner wurde die Reduction von einigen Autoren* nicht in der Entwicklungsphase der Genitalzellen gesucht, sondern in den Uebergangsstadien von der geschlechtlichen zur ungeschlechtlichen Generation.

Bei diesen Sachverhältnissen schien es mir sehr wünschenswerth zu sein, eine genauere Bearbeitung der pflanzlichen Genitalzellen vorzunehmen, um so mehr als die bisherigen Untersuchungen der Botaniker über diese Frage von einigen Zoologen† in Zweifel gezogen wurden. Solche Untersuchungen mit vielen einheimischen Monocotyledonen haben mich seit einigen Jahren viel beschäftigt, hauptsächlich aber mit der gemeinen Zwiebel (*Allium fistulosum*, L.), welche von April bis Ende Juni häufig cultiviert wird. Vor allem zog ich diese Pflanze desshalb vor, da die Zahl und die Gestalt der Chromosomen mir sehr geeignet für solche Untersuchungen schienen, was sich in der Folge auch gezeigt hat.

Methode.

Die einfachste Methode zur Untersuchung der Pollenkörner besteht in der Zerzupfung der Antheren auf dem Objectträger, Fixierung und Färbung derselben. Diess geschieht sehr leicht unter einem Präpariermikroscope mittelst Nadeln, da der zähflüssige Schleim der Antherenkammern die Pollenzellen in wünschenswerther Weise auf dem Objectträger aufkleben lässt, so dass die Fixierung und Färbung bequem unternommen werden kann. Als einfachste Fixierungs-und Färbungsflüssigkeiten habe ich die bekannte Methylgrün-Essigsäure

* Strasburger, (35).
† Haecker, (15,17).

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und SCHNEIDER's Essigsäurecarmin angewandt, welche beide in der Wirkungsweise ähnlich sind und sich sehr für das Studium der Chromosomen eignen. Sie verursachen aber starke Quellungen der ganzen Zellen sowie der Chromosomen; auch sind die Præparate nicht haltbar. Um Dauerpræparate zu bekommen habe ich viele Flüssigkeiten versucht: Alcohol-Essigsäure, mit oder ohne Zusatz von Sublimat; FLEMMING's Chrom-Osmium-Essigsäure, concentrirt, oder mit Wasser verdünnt; ½ % Chromsäure; VOM RATH's Picrin-Osmium-Essigsäure-Gemisch; Platinchlorid-Essigsäure; Ameisensäure und a.m. Von allen diesen habe ich aber den besten Erfolg gehabt mit den verdünnten Lösungen VOM RATH's und FLEMMING's und mit Alcohol-Essigsäure. Die Objectträger mit den Pollenkörner kommen entweder in eine schwache FLEMMING's oder in stark verdünnte VOM RATH's Lösung, etwa für 10 Sekunden, oder in eine Lösung von Alcohol absolutus und Essigsäure zu gleichen Theilen für drei bis fünf Sekunden, oder nur momentan in eine Lösung von Alcohol absolutus mit Essigsäure und Sublimat. Hierauf wurden die Objecte sorgfältig mit Wasser gewaschen, dann allmählig durch Alcohol verschiedener Concentration gehärtet. Als Färbungsflüssigkeit wandte ich Alaun-Carmin, Carmalaun, GRENACHER's Hæmatoxylin, Hæmalaun nach PAUL MAYER, sowie verschiedene Anilinfarben an. In Essigsäure-Methylgrün, Essigsäure-Carmin, oder in Essigsäure-Alcohol lassen sich die achromatischen Fadenstrukturen mit Ausnahmen von sehr wenigen Fällen schwer oder gar nicht sehen, wogegen dieselben in FLEMMING'schen oder in vom RATH'schen Lösungen sehr deutlich hervortreten.

Ausser diesen Zupfpræparaten, die für die Zählung der Chromosomen unentbehrlich sind, habe ich auch Schnitte angefertigt. Zu diesem Zweck wurden die Antheren oder die ganze Blüthenknospen in 1 % Chromsäure, in starker FLEMMING's Lösung, in Picrin-Essig-

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Osmium-Säure VOM RATH's oder in Alcohol absolutus eingelegt, und die Schnitte mit verschiedenen Färbungsflüssigkeiten behandelt.

A. Eigene Beobachtungen.

1. Die Chromosomenzahl in den Gewebszellen von Allium Fistulosum, L.

Karyokinetische Figuren sind hier in verschiedenen Gewebszellen der Pflanze leicht zu beobachten, besonders in den Epidermiszellen der Blüthenknospen. Die mitotischen Figuren dieser Zellen zeigen uns keine Abweichungen von den gewöhnlichen Modi. Die jungen Knäuelformen zeichnen sich wie sonst aus durch eine scheinbar continuirliche zusammenhängende Schleife, in welcher die Längsspaltung manchmal deutlich zu sehen ist. Die Schleife zerfällt nun in sechszehn Chromosomenzwillinge von ungefähr gleicher Länge. Diese liegen zuerst offenbar ganz unregelmässig im Kernraum zerstreut ; allmählig aber ordnen sie sich am Equator der inzwischen geformten Spindel. Die sechszehn Chromosomenpaarlinge wurden nun von einander getrennt, ganz in gewöhnlicher Weise wie es FLEMMING (8) angiebt. Eine solche Zelle am Schlusse der Metakinese findet man in Fig. 1 repræsentiert. Alle anderen Gewebszellen zeigen auch die sechszehn Chromosomen wie in dieser Zelle, und in Uebereinstimmung mit den Angaben STRASBURGER's (33) von *Allium odorum*.

2. Urpollenzelle.

Die Abtrennung der Urpollenzelle von den Zellenreihen der befruchteten Eizelle konnte ich leider nicht auffinden. Die jüngste Genitalzelle findet man im Innern der ganz kleinen Antherenanlage, welche als fünf schwache Erhebungen am oberen Rande der Blüthenknospe zu bemerken sind. Eine solche Zelle zeichnet sich durch ihre relative Grösse des Zellkerns, sowie durch die Chromatinarmuth des

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letzteren aus. Figuren 2, 3, und 4 zeigen diese Zellen. Dieselben wurden mit FLEMMING's Lösung fixiert und mit Methylenblau-Fuchsin gefärbt.

Das Cytoplasma zeigt bei guter Beleuchtung feine netz-oder schaumige Structuren, die durch Fuchsin schwach gefärbt sind. Der Kernraum ist von Lininsträngen durchsetzt, auf welchen feine Chromatinkörner ganz regelmässig zerstreut sind. Diese letzteren sind sehr klein in den jüngeren Zellen, werden allmählig grösser in älteren Stadien (Fig. 2, 3, und 4). Die Kernkörperchen liegen in den meisten Zellen etwas excentrisch. Dieselben sind oval oder länglich-oval ; ganz kugelrund findet man sie sehr selten vor. Ihre Zahl beträgt ein oder zwei. In vielen Fällen beobachtet man einen freien Raum um das Kernkörperchen (Fig. 2). Bei der Farbenreaction dieser Struckturen bemerken wir, dass das Chromatin Methylenblau aufnimmt, während das Kernkörperchen sehr stark mit Fuchsin gefärbt wird. Auch das Linin lässt sich mit Fuchsin färben, wenn auch viel schwächer wie das Kernkörperchen.

In etwas späteren Stadien findet man die Nucleolen stark verkleinert, während das Maschenwerk des Linins viel grösser wird. Auch die Chromatinkörner sind nun viel grösser geworden und nehmen kugelige Gestalt an (Fig. 5). Der Nucleolus ist dagegen stark verkleinert, so dass derselbe in den mit Hæmatoxylin gefärbten Præparaten kaum von den Chromatinkörnern zu unterscheiden ist. In Methylenblau-Fuchsin Præparaten kann man aber den mit Fuchsin roth gefärbten Nucleolus von den blauen Chromatinkörnern sehr deutlich unterscheiden. Diese Verkleinerung des Nucleolus ist offenbar durch allmälige Spaltung in kleinere Stückchen zu erklären, denn man beobachtet in solchen Kernen viele kleinere cyanophile Körperchen, die nur als zerstückelte Nucleolen zu deuten sind, wie bereits von vielen Botanikern vermuthet wurde.

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Nun nähern sich die Chromatinkörner mehr und mehr und bilden einen viel gewundenen Strang von perlschnurartigem Aussehen. Die einzelnen Chromatinkörnchen dieses Stranges haben aber keine glatte Oberfläche ; dieser sendet, wie gewöhnlich in diesem frühen Knäuelstadium zu beobachten ist, zahlreiche Fortsätze strahlig aus. Der Chromatinstrang zieht hierauf allmählig diese Fortsätze wieder ein und wird wieder glatt wobei seine Dicke etwas abnimmt (Fig. 6). Die achromatischen Stränge der Kerne sind in diesem und in den folgenden Stadien nicht mehr so deutlich zu sehen wie früher. Die nächsten Stadien zeignen sich durch den Zerfall des Strangs in Segmente aus, deren Zahl aber bei ihrem ersten Auftreten wegen ihrer zahlreichen Windungen nicht genau zu bestimmen sind. In Fig. 7 und 8 habe ich zwei Zellen in diesem Zustande gezeichnet ; ich muss aber gleich hier bemerken, dass dieselben von einem ziemlich dicken Schnitt stammen, so dass die Kernsegmente in grösserer Zahl vorhanden zu sein scheinen als es in der That der Fall ist. Die Kernkörperchen sind hier noch vorhanden und reagieren cyanophil. Nun fängt die Verkürzung der einzelnen Chromosomen an, wobei ihre Zahl deutlicher hervortritt. In Fig. 9 sieht man eine solche Zelle mit sehr kurzen Chromosomen dargestellt. Diese betragen zwar acht aber nur sieben sind an der Figur zu sehen, da einer der in dem vorhergehenden Schnitte lag nicht mitgezeichnet wurde. Diese dicken Chromosomen sind nicht ganz gleichmässig gebaut, indem viele derselben verdickte stark tingierbare Enden zeigen. Die Nucleolen scheinen in diesem Stadium völlig verschwunden zu sein ; ihr Schicksal ist mir dunkel geblieben.

Jetzt tritt eine Längstheilung der Chromosomen ein (Fig. 10). In manchen findet aber keine völlige Abtrennung statt, sondern Schwester-Chromosomen haften sich mit ihren Enden

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an einander (Fig. 11), und bleiben so bis zur Metakinese, wie es in FLEMMING's heterotypischer Theilung der Fall ist. Auch im Monasterstadium sind die Chromosomen nicht regelmässig angeordnet. Sie werden in den Metaphasen sehr lang gezogen (Fig. 12, 13, 14, u. 15), deren Achtzahl dabei sehr deutlich hervortritt, besonders wenn man dieselbe von den Polen aus betrachtet, wie aus den Fig. 14 und 15 ersichtlich ist. An den Polen angelangt fangen die Chromosomen an sich wieder zu verkürzen, wie gewöhnlich (Fig. 16 u. 17), was in der Bildung von ruhenden Kernen endet (Fig. 18). In dem Stadium, das wir in Fig. 18 darstellten, findet man die Bildung der Zellplatte. Die Chromosomen werden wie sonst aufgelockert, aber behalten ihre polare Anordnung bis zur völligen Abtrennung beider Tochterzellen bei (Fig. 19 u. 20). Die cyanophil sich färbenden Nucleolen, welche entweder einzeln oder zu zweien vorhanden sind, können erst im Stadium von Fig. 18 beobachtet werden, und haben sich im Stadium von Fig. 19 beträchtlich vergrössert.

Die Zellen, die ich in Fig. 1 bis 20 gezeichnet habe, kommen nicht in einer und derselben Antherenanlage vor. Das in Fig. 2 bis 5 dargestellte Stadium wurde wie gesagt nur in sehr jungen Antherenanlagen gefunden, während die folgenden von weiter entwickelten, aber noch ungestielten Knospen entnommen sind. Auch sind diese letzteren von verschieden Antheren abgezeichnet, so dass ihre relative Grösse nicht nach diesen Figuren zu beurtheilen ist. Die Grösse der Zellen nimmt ganz regelmässig von Fig. 2 bis zu Fig. 8 zu, aber da die in den Figuren 9, 10, 17, 18, 19, u. 20 dargestellten Zellen von anderen Antheren entnommen sind, und auch mit anderen Flüssigkeiten conserviert wurden als diejenigen der übrigen Figuren, scheinen sie verhältnissmässig klein.

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3. Pollenmutterzellen.

Die letzten Theilungen der Urpollenzellen liefern uns die Pollenmutterzellen. Die jüngsten Stadien einer solchen findet man in Fig. 19 und 20 angegeben. Die ziemlich groben Chromatinelemente dieser Zellen werden gleich wieder ganz fein im Kernraum vertheilt und wir bekommen nun wieder ähnliche Strukturen wie wir oben bei den Urpollenzellen beschrieben haben. Die Nucleolen dieser Zellen sind auch in der Ein- oder Zweizahl vorhanden, ferner sind sie nicht ganz rund, sondern in vielen Fällen oval oder elliptisch. Fig. 21, 22, u. 23 zeigen diese Zellen in dem sogenannten Ruhestadium. Auf dem sehr feinen Lininnetz sind die Chromatinkörner fast gleichmässig vertheilt. Diese Zellen nehmen an Volumen stetig zu, wobei der Kern verhältnissmässig mehr als der Zellkörper wächst. Die gleichmässig zerstreuten Chromatinstränge werden allmählig um die Nucleolen gruppiert (Fig. 24, 25, u. 26). Auch sind die Chromatinkörner grösser geworden, aber bei weitem nicht so gross, wie in der correspondierenden Stadien der Urpollenzellen (vergleiche Fig. 3, 4, u. 5 mit Fig. 24, 25, u. 26). In diesen und in folgenden Stadien sieht man die wabigen Strukturen des Cytoplasma sehr deutlich, wo die Præparate mit FLEMMING's oder mit VOM RATH's Flüssigkeiten fixiert sind.

Die Lininfäden, auf denen die Chromatinkörner sitzen, scheinen allmählig mehr umzubiegen und es entsteht endlich ein viel gewundener Strang (Fig. 27 u. 28). Ganz sicher kann man aber nicht sein, ob der Strang ein continuirtes Gebilde ist, oder ob er bereits aus vielen Segmenten besteht. Ich neige aber zu der ersten Alternative, denn man kann keine freien Enden des Strangs erkennen. Freilich kann man dies in Schnitten nicht entscheiden, wie in den Zellen, die ich in den Fig. 27 u. 28 dargestellt habe ; aber wir finden

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auch in Zupfpræparaten keine Strang-Enden. Die Chromatinkörner sind nun viel grösser geworden ; sie ordnen sich aber noch in einfacher Reihe auf den Lininfäden an. Man findet noch in diesem Stadium ein oder zwei Nucleolen, die entweder ganz nahe nebeneinander liegen, oder auch in einiger Entfernung von einander. Diese Stadien zeichnen sich durch die Zusammengruppierung des Chromatinstrangs um die Nucleolen aus, so dass in vielen Kernen, wo der oder die Nucleolen an einer Seite des Kernraums zu finden sind, der Kerninhalt nur am dieser Seite zusammengerückt scheint, ein Zustand, welchem Moore (24) den Name " synapsis " gegeben hat, und der von vielen Forschern in ähnlichen Entwicklungsstadien der Genitalzellen verschiedener Organismen beschrieben worden ist. Ganz allmählig wird der Chromatinstrang regelmässig im Kernraum gestreckt, und nimmt dabei das Volumen stetig zu (Fig. 29–35). In Fig. 29 u. 30 sehen wir keine Nucleolen, da die Zellen mit Essigsäure-Carmin behandelt sind ; diese finden wir aber in allen andern Zellen dieses Stadiums. In Figur 31 rechts und in Fig. 32 unten finden wir ein oder zwei kleine stark mit Fuchsin tingierte Körperchen im Cytoplasma, die wir für die Centrosomen halten dürfen. Die Chromatinkörner des Strangs haben auch wie gewöhnlich keine glatte Conturen, sondern viele Fortsätze (Fig. 32).

Die Segmentation der Chromosomen scheint in diesen oder in den gleich darauffolgenden Stadien stattzufinden, denn wir finden schon in den folgenden Stadien eine Anzahl von vereinzelten Chromosomen. Diess sehen wir in den Fig. 36 u. 37 dargestellt. Die Chromatinkörner der Stränge werden nun in zwei Reihen angeordnet, was vermuthlich durch Theilungen herbeigeführt wird. So finden wir in diesen Stadien den ersten Anfang zur Längstheilung der Chromosomen. Die Zahl der Chromosomen lässt sich aber noch nicht mit Sicherheit feststellen, denn sie sind noch ziemlich lang. Nach dem letzten Stadium

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findet die Längsspaltung der Chromosomen statt. Immerhin sind einige von ihnen nicht durchgespalten, wie man in der Zelle, Fig. 38 rechts oben, sieht. Ihre Lage im Kernraum zeigt noch keine Regelmässigkeit an. Viele der Paare nehmen nun eine ganz eigenthümliche Gestalt an, indem sich eine starke Knickung an der mittleren Partie einstellt, und so sich ein mehr oder minder gut ausgeprägtes V-Figur (Fig. 39 und 40) ausgestaltet. Die einzelnen Mikrosomen in diesen Chromosomen kann man wegen der starken Tinctionsfähigkeit der letzteren nicht beobachten. Jene Paare verkürzen sich allmählig, und die Mikrosomen kommen wieder zur Erscheinung. Diese sind nun viel grösser geworden wie früher, auch ihre Gestalt ist nicht mehr eine flache Scheibe, sondern kugelig (Fig. 41 bis 44). Ihre Zahl ist jedoch sehr schwer zu bestimmen, doch lässt sie sich in vielen Fällen auf vierzehn schätzen. Kleine cyanophile Körperchen werden auch in vielen Zellen dieser Stadien angetroffen (Eig. 38, 39, u. 41). Ob sie jedoch den Centrosomen entsprechen, was sehr wahrscheinlich ist, habe ich nicht entschieden. Man findet noch in diesen Stadien wohl entwickelte Nucleolen, die aber in den Fig. 43 u. 44 nicht angegeben sind.

Die Verkürzung der Chromosomen schreitet dann noch weiter fort, und die Mikrosomen werden wieder unsichtbar. Es findet jetzt eine wichtige Veränderung der Chromosomen statt. Die Paare verknüpfen sich an ihren Verknickungsstellen mit einander, und es entsteht so eine Chromosomengruppe, die mit den in den Entwicklungscyclen der thierischen Genitalzellen so allgemeinen beobachteten Vierergruppe einige Aehnlichkeit hat. Diese finden wir in Fig. 45–70 dargestellt.

Figuren 45–47 wurden von einem Schnittpræparat entnommen ; die paarigen Chromosomen lassen sich hier nicht deutlich beobachten.

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Auch ist die Zahl der Chromosomen in diesen Figuren nicht zu erkennen. Man sieht die Nucleolen und die Kernmembran noch sehr deutlich, obwohl die ersteren jetzt in Färbungsflüssigkeiten nur sehr blasse Farbe annehmen. Diese beiden Strukturen werden aber bald darauf aufgelöst, und hiermit entsteht jetzt eine zierliche Spindel, die in Essigsäure- Præparaten jedoch nicht oder sehr undeutlich gesehen werden kann. So sind die in den Fig. 48–55 dargestellten Spindeln, welche mit Alcohol-Essigsäure behandelt wurden, sehr undeutlich, und in den mit Essigsäure-Carmin behandelten Præparaten von Fig. 62–70 fast gar nicht zu sehen, wogegen in den Fig. 56–61 die Central- sowie die Mantelfasern mit aller Deutlichkeit hervortreten. Diese letzteren Figuren wurden nach Præparaten gezeichnet, die wir mit FLEMMING's concentrirter Flüssigkeit behandelt haben. Dieselben zeigen auch die natürliche Grösse, während die anderen entweder zu stark aufgequollen (Fig. 62–70) oder ein wenig geschrumpft sind (Fig. 48–55). Die Essigsäure scheint übrigens nicht mit gleicher Intensität auf das Cytoplasma wie auf die Chromosomen zu wirken, denn die letzteren Structuren behalten beinahe dieselbe Grösse in Essigsäure-Præparaten wie in denen mit FLEMMING's Flüssigkeit behandelten (vergleiche die Fig. 53–55, 56–61 mit Fig. 62–70).

Endlich nehmen die Chromosomen eine equatoriale Stellung ein, ohne dabei wie in gewöhnlicher Mitose im Kranze angeordnet zu sein. Einzelne Paare lassen sich im Inneren der Spindel erkennen, besonders deutlich bei Polansicht, wobei oft klar hervortritt, dass die freien Schenkelenden der geknickten Chromosomen nach Aussen gerichtet liegen. Was die Lage der einzelnen Chromosomen in einem Paarling betrifft, so liegen dieselben übereinander in der Aequatorialplatte wobei der eine dem Südpol und der andere dem Nordpol zugekehrt ist. In fast schematischer Schönheit sieht man diese

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Lagerung in den Seitenansichten der Fig. 52, 64, u. 70. Anders gestellte Paarlinge lassen sich jedoch immerhin in vereinzelten Fällen beobachten.

Die oben beschriebenen Vorgänge spielen sich während des Monasterstadiums ab. Von jetzt fängt die Metakinese an, und führt zur Bildung der Pollentochterzellen. Diess ist in den Fig. 71–88 veranschaulicht. Die Schwesterchromosomen werden nun von einander getrennt. Dies geschieht mehr nach den homöotypischen als nach den heterotypischen Modi. Alle Schwesterchromosomen werden jedoch nicht auf einmal von einander getrennt, sondern einige davon erst nach längerer oder kürzerer Zeit (Fig. 71–74). Auch werden sie nicht so lang gezogen wie in den Metakinesen der Urpollenzellen, sondern bleiben kurz und dick (vergleiche Fig. 12–15 mit 71–74). In diesem Metakinesenstadium treten nun viele cyanophile Körperchen im Zellraum auf, meistens auf den Spindelfasern liegend, aber auch im übrigen Cytoplasma, wie sie von anderen Autoren in verschiedenen Zellarten schon beobachtet und beschrieben sind. Diese ordnen sich offenbar an der Equatorialebene an, gegen das Ende der Metakinese, um die Zellplattenkörner zu bilden, gerade so wie sie von VON KOSTANECKI (23) in den Embryonalzellen von *Kaninchen* beobachtet worden sind.

Die acht V-förmigen Tochterchromosomen welche an beiden Polen der Spindeln sich angesammelt haben, verkürzen sich nun mehr, und es beginnt nun ein ganz interessanter Vorgang. Die V-förmigen Chromosomen brechen an ihrer Verknickungsstelle durch, und so entstehen sechszehn stäbchenförmige Enkelchromosomen. Veranschaulicht werden diese Stadien in den Fig. 79–85. Die Chromosomen in Fig. 81 behielten noch ihre V-Gestalt. Im Fig. 80 aber sieht man die zwei Chromosomen an der rechts liegenden Gruppe schon deutlich getrennt und haben sich um

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90 Grade gedreht. Solche abgetrennte Chromosomen finden wir auch in den Fig. 82 u. 83. Am schönsten sieht man sie in Fig. 84, wo alle Chromosomen die völlige Trennung zeigen. Auch die Zahl der Chromosomen ist nun leicht zu erkennen. In der oberen Gruppe können wir die acht Paare genau constatiren, während in der unteren zwei Paare an der linken Seite als ein Stäbchen zur Anschauung kommen. So finden wir am Ende der Metaphasen acht Chromosomenpaarlinge oder sechszehn einzelne Chromosomen an jedem Pole der Zelle. Diese Verdoppelung der Chromosomen an Dyastern hat eine auffallende Aehnlichkeit mit dem was FLEMMING in der heterotypischen Kerntheilung an den Samenzellen von *Salamandern* nachgewiesen hat, obwohl die Theilungsvorgänge in beiden Fällen recht verschieden sind. Ich komme auf diese Frage im allgemeinen Theil wieder zurück. Schon an dieser Stelle sei aber so viel bemerkt, dass die paarigen geknickten Chromosomen nicht, wie in FLEMMING's Fall, Längsspaltung erfahren, sondern Quertheilung. Die Chromosomen verkürzen sich nun noch mehr, bis man ihre Individualität nicht mehr erkennen kann (Fig. 85, 86). Bald werden sie aber nochmals von einander gelöst und enden in der Bildung von Tochterkernen (Fig. 78, 87, 89 u. 90). Während dieser Vorgänge wird die Zellplatte gebildet und die Pollenmutterzelle wird in die Pollentochterzellen getheilt.

4. Pollentochterzelle.

Wenn die Chromosomen in den beiden Tochterzellen wieder sichtbar werden, finden wir diese nicht in vereinzelten Stäbchen wieder, sondern in Paaren vereinigt. Die Zahl dieser Paarlinge beträgt acht. Sie vereinigen sich aber, nicht wie in den Pollenmutterzellen, an ihren mittleren Theilen, sondern an ihren beiden Enden, so dass ringförmige Chromosomen entstehen, gleich wie es bei

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der heterotypen Metakinese des *Salamanders* der Fall ist. Veranschaulicht werden diese Stadien in Fig. 87 bis 107.

In Fig. 87 sind die Chromosomen noch mit einander verbunden, so dass sich die einzelne Ringe noch nicht mit Sicherheit herausstellen. Auch die Chromosomen sind noch recht dick. Dieselben sind aber in Fig. 88 etwas dünner geworden, und es lassen sich in dieser Zelle einige ringförmige Anordnungen der Chromosomen erkennen ; so z. B. in der oberen Gruppe. Gleichfalls kann man das auch von den Zellen von Fig. 89 u. 90 sagen, wo die Kernmembran schon aufgetrieben ist. In dieser Zelle sind die kugeligen Mikrosomen wieder aufs deutlichste zu beobachten, aber die ringförmigen Chromosomen kann man hier noch nicht deutlich sehen ; anderseits findet man in einem etwas weit fortgeschrittenem Stadium (Fig. 78) die Chromosomen fast in Mikrosomenkörnern zerlegt, ganz so wie wir es in den Anaphasen der Urpollenzellen gesehen haben (vergleiche Fig. 18). Dasselbe findet auch in der Zelle von Fig. 91 statt. In seltenen Fällen begegnet man einer noch feinerer Zerlegung der Chromatinkörner als in Fig. 91 angegeben ist. Immerhin aber behält der Kern seine polare Anordnung der Chromosomen bei , d. h. das Polfeld RABL's ist immer zu bemerken. In weiteren Stadien findet man die ringförmigen Strukturen der Chromosomen mehr und mehr gut ausgeprägt, (Fig. 92–98). In Fig. 92 sehen wir die Chromatinkörner etwas mehr zusammengenähert wie in Fig. 91, und in Fig. 93 noch mehr. Man sieht in Fig. 93 die Chromosomen in dem oberen Kerne zu engen Reihen angeordnet, und mit ihren Enden untereinander verbunden. In den übrigen Figuren (Fig. 94–97) tritt die Ringform noch deutlicher hervor ; so z. B. in Fig. 94, 95, oder 96.

Während dieser Veränderungen, nimmt die Tinktionsfähigkeit der Chromosomen mehr und mehr zu, und die einzelnen Mikrosomen werden wieder sichtbar (Fig. 98–107). Auch die Chromosomen

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ändern ihre Anordnung im Kernraum, und das darauf folgende Monasterstadium ist regelmässiger, wie dasjenige in der Pollenmutterzelle (Fig. 97–100, und Fig. 113 rechts). In Fig. 98, wo man nur eine von den zwei Zellen in Polansicht beobachtet, sieht man die acht Chromosomenringe zu einem Kranz angeordnet. Hier oder in der rechten Zelle von Fig. 113 bemerkt man eine auffallende Aehnlichkeit mit dem nämlichen Stadium der heterotypischen Form der Hodenzellen von *Salamander* wie es in Fig. 24 Taf. XXIV von FLEMMING's „Neue Beiträge (8)" dargestellt ist. Der Kern verliert jetzt seine Membran, und das Cyto- und Nucleoplasma kommen in Berührung mit einander. Gleichzeitig mit dem Verschwinden der Kernmembran erscheinen die Spindelfasern (Fig. 105–107). Die Chromosomengruppe nimmt nun an Umfang bedeutend zu (vergleiche Fig. 97, 98 mit Fig. 99–102).

Die Theilung der Pollentochterzellen schreitet nach dem heterotypischen Modus vor (Fig. 103–107). Die Tochterchromosomen bleiben längere Zeit beisammen, dabei nehmen die Chromosomenringe recht verschiedenartige Gestalt an. Man sieht auch in einigen Fällen eine reguläre Anordnung, wie wir das in Fig. 106 dargestellt haben. Dieselbe Zelle zeigt noch eine weitere Abweichung darin, dass einige Paare in diesem Stadium bereits völlig getrennt sind. Aber gerade diese Zelle zeigt uns noch die interessante Thatsache, dass die Trennung des Chromosomenrings in der Vereinigungsstelle der Tochterchromosomen stattfindet.

Die acht Chromosomenringe dieses Monasters werden nun in zwei Gruppen von acht einfachen Chromosomen getrennt. Der Habitus dieser Chromosomen ist anders als derjenige in den Urpollenzellen. Diese Metakinesestadien finden wir in Fig. 109–117 dargestellt. In Fig. 109 und 110 sind einige Tochterchromosomen noch nicht völlig von einander getrennt.

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Vergleicht man diese Chromosomen mit denen der Fig. 71–77, so wird man sofort sehen, dass sie von einander recht verschieden sind, wie der Vergleich mit den Fig. 12–17 ergibt. Diese Verhältnisse verdienen weitere Beachtung, indem hier wie in der Urpollenzelle ein Ruhestadium folgt, während die Pollenmutterzelle sich gleich wieder zu Theilung anschickt.

Die Umwandlung der Enkelkerne in Anaphasen ist ähnlich wie in den Prophasen, nur schreitet sie dort weiter vorwärts wie hier. In ihren ersten Umwandlungsstadien sieht man die einzelnen Mikrosomen wieder deutlich (Fig. 113–117), und auch ist das RABL's Polfeld wohl zu erkennen. Die Theilungsflächen der beiden Mutterzellen befinden sich nicht immer in derselben Ebene, sondern ihre Richtungen sind recht verschieden, wie man aus den Fig. 105, 107, 110, 111, 116, u. 117 sehen kann. Wenn die Nuclei das Ruhestadium erreicht haben, dann platzt die gemeinsame Membran, und die einzelnen Pollenzellen treten nun heraus.

5. Pollenzelle.

Der Kern macht noch eine weitere Unwandlung durch, und kehrt zu seinem völligen Ruhezustand zurück. Das Chromatinnetz bildet sich vollständig so wie in der Urpollenzelle. Es entsteht gewöhnlich ein grosser Nucleolus. Bald aber werden die Chromatinkörner gröber und eine Anzahl von Chromosomen tritt wieder zu Tage. Jedoch sind die Chromosomen noch sehr lang und sehr gewunden, so dass man ihre Zahl nicht gut erkennen kann. Die Chromosomen sind aber noch nicht doppelt, d. h. es giebt kein Anzeichen von einer Längstheilung in diesem Stadium wie man aus den Fig. 119 u. 120 gut sehen kann. Nun werden die Mikrosomen wieder sichtbar (Fig. 121), und bald darauf werden jene der Länge nach getheilt (Fig. 122).

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Nun fangen die Chromosomenpaare an sich zu verkürzen, und ihre Achtzahl wird wieder sichtbar (Fig. 122–125). Die Längstheilung scheint aber nicht gleichzeitig in den sämmtlichen Chromosomen stattzufinden, wie man an Fig. 128 sieht. Auch in diesem Stadium zeigen die Tochterchromosomen eine Neigung an ihren Enden zu verschmelzen, und so entstehen wieder Chromosomenringe (So z. B. eine an der linken Seite der Fig. 130). Kurz nach ihrer Längstheilung biegen sich die Chromosomen an ihren centralen Theilen um, gerade so wie es in der Pollenmutterzelle stattfand; aber nicht so stark wie in diesem Falle, so dass sie gewöhnlich eine U- und nicht V-förmige Gestalt annehmen.

Wenn die Längsspaltung der Chromosomen vollendet ist, so tritt das Monasterstadium ein. Dieses ist aber nicht nach dem gewöhnlichen Typus gebildet, sondern mehr nach dem heterotypen.

Spindeln und Zellplatte habe ich erst im Diasterstadium beobachtet (Fig. 134, 135). Fig. 136 zeigt ein Stadium gleich nach der Abtrennung des vegetativen Kerns. Dieser liegt aber gerade unter dem generativen und ganz von dem letzteren verdeckt, was wir in dem Bilde nicht angegeben haben. Die Chromosomen verkürzen sich in gleicher Weise in beiden Kernen. Mit ihrem Lockerwerden tritt aber eine erhebliche Differenz zwischen den beiden Kernen ein (Fig. 137 v. 138). Der vegetative Kern verkleinert sich bald und nimmt dabei einen intensiveren Farbenton an, als der generative, etwa so wie wir zwischen den Kernen der Keim- und Nährzellen vieler Thiere beobachten. Viel stärker färbt sich aber das grosse Kernkörperchen des generativen Kernes als das viel kleinere des vegetativen.

Auch in chemischer Hinsicht scheint eine Differenz zwischen diesen beiden Kernen zu bestehen, denn bei der Doppelfärbung einer Pollenzelle mit Säurefuchsin und Methylenblau zeigt der Kernsaft

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der generativen Zelle eine starke Neigung, die rothe Farbe anzunehmen, während derjenige der vegetativen die blaue.

Die Theilung des generativen Kernes im Pollenschlauche habe ich nicht beobachtet.

B. Zusammenfassung.

1. Die Gewebszellen von *Allium fistulosum*, L. enthalten sechszehn Chromosomen (Fig. 1).

2. Die jüngste Pollenzelle (Urpollenzelle) hat eine oder zwei ovale Nucleolen von beträchtlicher Grösse, und ein feines Chromatinnetz (Fig. 3, 3 u. 4).

3. Das Chromatinnetz wird allmählig gröber und das oder die Nucleoli kleiner (Fig. 5).

4. Im nächsten Stadium sammeln sich die Chromatinkörner zu einem viel gewundenen zusammenhängenden Netz, welches zuerst pseudopodienähnliche Fortsätze an der Oberfläche trägt; diese werden aber bald zurückgezogen (Fig. 6).

5. Nun tritt eine Segmentierung des Chromatinstranges ein. Die Segmente sind aber bei ihrem ersten Auftreten lang und viel gewunden, so dass ihre Zahl nicht zu erkennen ist (Fig. 7 u. 8).

6. Die Segmente verkürzen sich und die acht Chromosomen kommen nun zur Erscheinung (Fig. 9). Die Nucleoli verschwinden dabei.

7. Die Chromosomen verlängern sich wieder, und ihre Längsspaltung tritt jetzt ein (Fig. 10).

8. Die Metakinese findet nach dem heterotypen Modus statt (Fig. 11).

9. Nach der Theilung geht die Tochterzelle (die ich Pollenmutterzelle nenne) zu völligem Ruhestadium über (Fig. 21–23).

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10. Die regelmässig im Kernraum zerstreuten Chromatinkörner ordnen sich allmählig zu einem langen Strange zusammen (Fig. 24–27).

11. Die Mikrosomen werden unsichtbar, und die Segmentierung des Chromatinstranges tritt wieder ein.

12. Die Mikrosomen treten nun wieder auf (Fig. 32–35).

13. In den folgenden Stadien werden die Mikrosomen der Länge nach getheilt. Die Theilhälften sind aber mit einem Achromatinsubstanz verbunden (Fig. 36 u. 37).

14. Die Chromosomen erleiden eine starke Verkürzung, und die einzelnen Mikrosomen werden wieder unsichtbar. Nun tritt die Längsspaltung der Chromosomen ein (Fig. 38 u. 39).

15. Jedes Chromosoma knickt sich am centralen Theile um. In einzelnen Fällen kommen die Mikrosomen wieder zur Erscheinung (Fig. 44).

16. Nun werden die Chromosomen stark verkürzt und die Mikrosomen werden wieder unsichtbar (Fig. 45–47).

17. Die Schwester - Chromosomen haften sich auf ihrer Verknickungsstelle ein, und so entsteht eine X-förmiger Figur (Fig. 48).

18. Die Nucleoli und die Kernmembran verschwinden jetzt, und das Monasterstadium tritt ein.

19. Die Theilung dieser Zellen schreitet ähnlich wie bei dem FLEMMING's homoeotypen Modus vor (Fig. 71–74).

20. Die acht V- förmigen Tochter- Chromosomen brechen nun auf ihrer Verknickungsstelle durch und so entstehen acht Chromosomenpaarlinge (Fig. 76–84).

21. In den Tochterkernen lösen sich die Chromosomen in ihrer Mikrosomenkörner auf, aber ein völligen Ruhestadium wie in der Urpollenzellen tritt in diesen Zellen nicht ein (Fig. 78, 87–90).

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22. Die Theilung der Pollenmutterzellen führt zu der Bildung der Pollentochterzellen.

23. In diesen Zellen treten die Chromosomen wieder in der Acht-Zahl auf. Diese nehmen aber nicht in Gestalt eines einfachen Stranges, sondern ringförmige Gestalt an.

24. Die Theilung dieser Zellen findet nach dem FLEMMING's heterotypischen Modus statt. Sie führt zur Bildung von vier Pollenzellen.

25. Der Kern der soeben gebildeten Pollenzellen geht zum völligen Ruhestadium über, indem sich seine Chromosomen wieder in Chromatinkörner auflösen und ein beträchtlicher Nucleolus erscheint nun in dem Kern.

26. Die Mikrosomen ordnen sich wieder zu einem langgewundenen Strang zusammen, welcher nach der Verlängerung nochmal Mikrosomenbestandtheile zeigt (Fig. 221). Bald darauf wird der Chromosomenstrang in Segmente zerlegt.

27. Die Längstheilung der Chromosomen tritt gewöhnlich gleich nach ihrer Segmentation ein (Fig. 122).

28. Jedes Chromosoma verkürzt sich wieder, und ihre Acht-Zahl wird wieder deutlich.

29. Die acht Chromosomenpaarlinge biegen sich an ihrem centralen Theile um, und so entstehen U-förmige Figuren.

30. Die Kernmembran verschwindet, und die acht Chromosomenringe bereiten sich zur Bildung der vegetativen Zellen vor, indem die Tochterchromosomen sich wieder von einander trennen.

C. Deutung der obigen Beobachtungen und Besprechung der Literatur.

Aehnliche Vorgänge, wie ich sie oben geschildert habe, wurden bekanntlich von vielen Botanikern an verschiedenen Pflanzen beo-

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bachtet. Besonders die characteristischen Gestalten, welche die Chromosomen in dem Theilungsstadium von Pollenmutterzellen annehmen, sind so auffallend, dass sie von allen Beobachtern, die sich mit der Entwicklungsgeschichte der Pollenkörner beschäftigt haben, sorgfältig beschrieben wurden. Das Gleiche wurde auch von SARGANT (32) neuerdings bei der ersten Theilung des Embryosack-Kernes von *Lilium Martagon* beobachtet und beschrieben (Fig. 19-22 auf Taf. XXIII). Der Entstehungsmodus dieser Figuren ist aber fast in allen anderen Fällen so compliciert, dass es sehr schwer ist, ihre Entstehung durch Längstheilung aus einzelnen Chromosomen zu verfolgen. Dass aber jene Autoren aus ihren Beobachtungen richtige Schlüsse gezogen haben, beweisen die oben beschriebenen Vorbereitungsprocesse zu der ersten Theilung der Pollenmutterzelle, und HAECKER (15) irrt sich hier, wenn er im Widerspruch zu STRASBURGER's (35) Verallgemeinerung folgendes äussert: "And so I believe if a true reduction of the chromosomes takes place anywhere in the generative cell- cycle of plants it must exist there" (d. h. in der ersten Theilung der Pollenmutterzellen). Ich muss aber gleich hier bemerken, dass was HAECKER unter einer Reduction versteht, nur in seinem (14,16) und in RÜCKERT's (30,31) Sinne gemeint ist, aber nicht im Sinne WEISMANN's (37,38) und VOM RATH's (29), indem die beiden letzten Autoren die beiden Theilungen der Samenmutterzelle (Spermatocyte) als Reductionstheilungen aufzufassen pflegen, während HAECKER (16) und RÜCKERT (31) nur die letztere. Wir haben aber in dieser Beziehung HAECKER zu verdanken, dass er zum ersten Male die auffallende Aehnlichkeit hervorhob welche zwischen den Vorbereitungsstadien zu den Theilungen der Pollenmutterzellen und denen der Spermatocyten- resp. Richtungskörper besteht.

In den genauer bearbeiteten Fällen betreffs den Ei- und Samenzelle finden wir nun in diesen Stadien die bekannte Vierergruppe,

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deren Entstehungsweise von verschiedenen Autoren verschieden aufgefasst wurde. Weil namentlich BOVERI (2) und BRAUER (4,5), denen sich auch MOORE (7,24) anschliesst, die Vierergruppe durch zweimalige Längsspaltungen eines primären Fadens entstehen liessen, denken sich HAECKER (16), VOM RATH (29), und RÜCKERT (31) sie durch eine Längs- und eine Quertheilung enstanden. BOVERI, der mit der Entwicklungsgeschichte des Eies des Pferdspulwurms sich länger beschäftigt hat, denkt sich, dass in dem Keimbläschen von *Ascaris megalocephala univalens* nur ein einziges Chromosoma vorhanden ist, welches durch zweimalige Längstheilungen in vier Stäbchen zerlegt wird. Diese vier Stäbchen wurden durch die Bildung von zwei Richtungskörperchen auf die ursprüngliche Einzahl reduciert. Das Gleiche wurde von BRAUER (4) bei der Bildung der Samenzelle desselben Wurms behauptet. Die Ursamenzelle von *Ascaris megalocephala univalens* enthält nach diesem Autor einen einzigen Chromatinfaden, welcher im Laufe der Entwicklung durch zwei Längstheilungen zu einem Bündel von vier Stäbchen sich umwandelt, von denen jedes zu einen Spermatid wird. Aehnliche Vorgänge wurden auch von HERTWIG (18) als wahrscheinlich hingestellt. Da aber die befruchtete Eizelle dieses Wurms zwei Chromosomen enthält, so denken sich BOVERI und BRAUER dass die Reduction schon im Keimbläschenstadium oder noch früher stattfindet.

HAECKER und RÜCKERT, die mit den Eireifungen von verschiedenen *Copepoden* sich beschäftigt haben, fanden auch die reducierte Zahl der Chromosomen bei diesen Keimbläschen ; aber sie halten diess wie gesagt nicht für eine wirkliche, sondern für eine scheinbare, denn die Chromosomen würden auffallender Weise eine Längs- und eine Quertheilung erleiden müssen, um die Vierergruppe zu bilden. Aehnliche Vorgänge wurden auch von VOM RATH in verschiedenartigen Thieren beobachtet, aber in schönster Weise in den

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Hodenzellen von *Salamandra maculosa* (27) und von *Gryllotalpa* (25,26), obwohl dieser Autor in der Deutung der Reduction von den beiden Anderen abweicht und die theoretisch postulierte Reduction WEISMANN's anzunehmen geneigt ist. Zu einem etwas abweichenden Resultat gelangte WILCOX (39,40) bei seinem Studium über die Samenbildung der *Caloptenus femur-rubrum*. Nach diesem Autor soll die Vierergruppe nach zwei Modi entstehen. Bei dem ersten Modus werden die Chromatinkörner in vierundzwanzig Punkte zusammengedrängt, und diese wiederum zu Vieren gruppirt, und so entstehen sechs Vierergruppen. Bei dem zweiten Modus wird der Chromatinstrang in zwölf Stücke segmentiert ; diese aber gleich in sechs Paare vereinigt, welche entweder so eng sich an einander lagern, dass die Paare als ein einziger Stab erscheinen, oder die Vereinigung ist sehr locker und giebt dem Paar den Anschein eines durch Längspaltung entstandenen Chromosomas. Die Chromatinkörner dieses Strangpaares verdichten sich zu je vier Chromosomengruppen. Durch zwei aufeinander folgende Theilungen der Spermatocyten werden die Chromosomen auf eines reduciert. Denken wir nun jedes der vierundzwanzig Chromatinkörner zu einem individuellen Chromosoma umgewandelt, so haben wir hier doppelt so viel Chromosomen wie in der befruchteten Eizelle ; werden aber jeder der zwölf Chromatinstränge zu einem Chromosoma, dann haben wir die Normalzahl. Das Auftreten der doppelten Zahl der Chromosomen in den Kernen der Spermato- respectiv Ovocyten ohne vorhergehende Längstheilung wie es hier von WILCOX beschrieben worden ist, steht in der Geschichte der Spermato- sowie Ovogenese der Thieren ganz vereinzelt da, obgleich es theoretisch nicht unmöglich, oder sogar sehr wahrscheinlich ist, und es bedarf deswegen noch eine weitere Bearbeitung als dies von WILCOX geschah. Das Auftreten einer Normalzahl von Chromosomen im Keimbläschen wurde auch von KORSCHELT (22) bei

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Ophryotrocha puerilis beobachtet und genau beschrieben. Auf botanischer Seite ist man aber vollständig überzeugt, dass sich beim ersten Auftreten der Chromosomen in den Pollenmutterzellen oder in den Kernen des Embryosackes eine auf die Hälfte reducierte Zahl zeigt. So fanden STRASBURGER (32–36), GUIGNARD (10), FARMER (6), und SARGANT (32) zwölf Chromosomen in Urkeimzellen der *Lilium Martagon* wo die Normalzahl vier-und-zwanzig beträgt. Jedes dieser zwölf Chromosomen theilt sich nach den Untersuchungen FARMER's (6,7) der Länge nach, wobei sich ein Ring oder eine am einer Seite unterbrochene acht-förmige Schleife oder auch zwei parallel laufende Schleifen sich bilden. Durch Verlötung der einen oder der beiden Enden dieser getheilten Chromosomen entstehen die bekannten T-, Y-oder auch X-förmigen Figuren. Diese Chromosomen werden nach diesen Autoren durch zweimalige Längsspaltungen auf die Tochterkernen vertheilt, so dass die Zahl der Chromosomen weder vermehrt noch vermindert wird. Es giebt also keine Reductionen der Chromosomen in diesen Stadien. Dieselben Vorgänge wurden auch von BELAJEFF (1) und STRASBURGER (36) für die Samenentwicklung von *Larix europea* behauptet. Nach STRASBURGER, dem die Reduction der Chromosomen in ganz anderem Sinne erscheint, wie WEISMANN, haben die complicierten Veränderungen welche die Chromosomen in den Vorbereitungsstadien zu den Theilungen bei den Pollenmutterzellen keinen Sinn, denn die Chromosomen-Reduktion wurde nach diesem Autor schon bei der Bildung der Archesporen vollendet.

Vergleicht man die Angaben und die Abbildungen dieser verschiedenen Autoren mit der oben geschilderten Pollenkörnerentwicklung von *Allium fistulosum*, so sehen wir dass ein wesentlicher Unterschied zwischen den beiden besteht. Während in *Lilium* und in *Larix* die durch Längsspaltung entstandenen Schwesterchromosomen

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gewöhnlich an ihren Enden vereinigt bleiben, trennen sich diese hier völlig von einander, so dass wir zwei parallel laufende Fasern bekommen, gerade so wie in den von FARMER als seltenerer Fall beschriebenen und in seinem schematischen Figur III C (7) angegebenen Doppelfäden. Besonders ähnlich sind sie mit den Chromosomen, welche wir im Keimbläschen vor den Reifungstheilungen thierischer Genitalzellen antreffen, wie namentlich mit denjenigen von *Cyclops signatus* nach HAECKER (14) oder von *Cyclops strenuus* nach Haecker (14,16) und nach RÜCKERT (30). Die paarigen Chromosomen unserer Pflanze zeigen ebenso wie in jenen Fällen stark verdickte Enden, die stärker durch Tinctionsmittel gefärbt werden als ihre mittleren Theile. Man vergleiche nur meine Fig. 39–42 mit RÜCKERT's Fig. 7 a, b und 8 a, b,c auf dessen Tafeln XXI und XXII, und ihre Aehnlichkeit ist ausser allem Zweifel.

Auch die weiteren Stadien dieser Gruppen erinnern uns mehrfach an die Bilder bei *Copepoden*, während das nächste Stadium eine auffallende Aehnlichkeit mit dem von *Salamandra maculosa* hat wie es von VOM RATH (27) in schönster Weise beschrieben wurde; man betrachte nur die Abbildungen 6 oder 7 auf dessen Taf. VII. Die V- förmigen Chromosomen unserer Pflanze zeigen ebenfalls verdickte Enden wenn auch nicht in so hohem Mass, wie diejenigen von *Salamandra*. Weil aber in *Salamandra* die Chromosomen gleich darauf sich in vier Kügelchen zerlegen, bleiben sie bei *Allium* zusammenhängend bis sie am Aequator der Spindel sich ordnen. Ob man aber diese Endverdickung der Chromosomen als das Streben zu ihrer Quertheilung ansehen darf, bleibt einstweilen unentschieden, da in älteren Stadien wo wir keine Quertheilung finden, man auch solche Chromosomengestalt (z. B. in Fig. 124, 125, 126, u. 131) antrifft. Jedenfalls lassen sich in vielen Chromosomen die verdickten Enden weit stärker tingieren als die mittleren Portionen, so dass man

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eine starke Ansammlung der Chromatinelemente an diesen Puncten annehmen darf.

Die Anordnung der Chromosomen am Spindeläquator, sowie die relative Lage der Doppelstäbchen ist ganz so wie diess von RÜCKERT (30) bei *Cyclops strenuus* beschrieben worden ist, d. h. der eine Stab liegt dem einen und der andere nach dem anderen Spindelpole zugewendet, so dass in Metakinese die Doppelstäbe an ihren Spalthälften von einander getrennt werden. Diess lässt sich hier aber wie gesagt nicht bei allen Paarlingen nachzuweisen, denn in vielen bleiben die Schwesterchromosomen an ihren mittleren Portionen so innig mit einander verbunden, dass ihre Individualität in vielen Fällen sich nicht erkennen lässt. Bei einigen Paaren findet man deutlich sogar andere Anordnung der Chromosomen z. B. in dem Paarlinge, welcher oben in der Zelle von Fig. 57 sind sie kreuzweise auf der Spindel angeordnet. Dasselbe sieht man auch in dem zweiten Paarlinge links in der Zelle von Fig. 65, dagegen in dem Paarlinge der an der rechten Seite dasselben Zelle liegt, befinden sich die einzelnen Chromosomen nebeneinander. In diesen Fällen, wenn keine Umordnung in ihrer Lage stattfindet, ehe sie sich von einander trennen, enthalten die nach dem Pole gelangten Tochter-Chromosomen andere Mikrosomencomponente als die ursprünglichen Spalthälften ; das hat viel Wahrscheinlichkeit für sich. Dass diese von theoretischer Standpunkte vom besonderen Interesse sind, braucht kaum hervorgehoben zu werden, da in dieser Weise diesbezüglichen Chromosomen werden der Quere nach getheilt, und so die Iden-Reduction WEISMANN's mit dieser Theilung in Vollzog gebracht werden kann, wenn man auch die Möglichkeit einer Umcombinierung der Iden in der Längstrennung der Chromosomen in Abrede stellt.

Aber noch ein interessantes Stadium wie ich oben schon angedeutet habe, spielt sich am Ende der Metakinese ab. Hier werden die acht

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V-förmige Chromosomen in sechszehn Stäbchen zerlegt, indem jedes V an der Kante zerbricht. Diese auffallende Erscheinung steht aber nicht ganz vereinzelt in der Geschichte der Genitalzellen. Bei *Cyclops strenuus*, wie von HAECKER (14) und RÜCKERT (30) angegeben wurde, findet diese Quergliederung der Chromosomen gewöhnlich viel früher statt. In vereinzelten Fällen sieht man aber diese sich erst im Dyasterstadium vollziehen. HAECKER (14) hat nämlich bei einigen Eiern dieses Thieres neben den schon getheilten und parallel laufenden Chromosomenpaare „winklig geknickte Stäbchen" im Dyaster der ersten Richtungsspindel gesehen und deutet sie, ganz richtig, als noch nicht getheilte Chromosomen. Während aber in thierischen Zellen diese Chromosomen mit dem gleich darauf folgenden zwei Theilungen sich auf vier Enkelzellen vertheilen, bilden sie sich bei unserer Pflanze zu ruhenden Kernen um, und lassen dann dabei ihre Individualität nicht mehr erkennen. In Anaphasen lässt sich aber feststellen, dass die Schwesterchromosomen allmählig an ihren freien Enden sich wieder mit einanden verlöthen, so dass eine mehr oder weniger gut ausgeprägte Kranzform entsteht. Ob aber die kranzförmig angeordneten Chromosomen, die wir in den Prophasen der nächsten Theilung beobachten die nämlichen oder ob diese Kränze Neubildungen sind, lässt sich hier natürlich nicht mit aller Bestimmtheit sagen. Immerhin würde es sonderbar erscheinen, wenn das letztere der Fall wäre. Wenn aber es sich als sicher herausstellt, dass die erstere Alternative der Wahrheit entspricht, was ich geneigt bin zu glauben, denn haben wir hier ganz ähnliche Vorgänge vor uns, wie in der Bildung des zweiten Richtungskörpers respective der letzten Theilung der Spermatocyten, wie sie von HAECKER, VOM RATH, und RUCKERT angegeben sind, und der zweiten Reductionstheilung WEISMANN's entsprechen.

So wichtig auch dieser Reductionsvorgang bei *Allium fistulosum* ist, so theile ich diess doch nur mit aller Reserve mit;

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denn wir finden in den sorgfältigsten Arbeiten über die nämlichen Gegenstände von GUIGNARD (10), STRASBURGER (33–36), BELAJEFF (1), FARMER (6,7), und SARGANT (32) keine ähnlichen Beobachtungen gegeben. STRASBURGER (36) der besonders diesem Punkte seine Aufmerksamkeit geschenkt hat, hebt nur zwei Längstheilungen von *Lilium* - Pollenmutterzelle hervor, womit auch FARMER (6) in seiner Arbeit über *Lilium*-Antheren einverstanden zu sein scheint. Das Gleiche wurde auch von SARGANT (32) in ihrer Oogenetischen Untersuchung für ähnliche Stadien beschrieben, obwohl diese Autorin nur sich speciell mit der Reductionsfrage beschäftigt hat.

Nehmen wir die Deutung der obengeschilderten Vorgänge bei *Allium fistulosum*, L. als richtig an, so werden die Chromosomenreduktionen entweder so erfolgen, dass die von VOM RATH postulierte Umcombinierung der Iden in den Längsspaltungen der Chromosomen bei der Mutterzelle erfolgen, oder dass diese Theilung nur einer Aequationstheilung entspricht. Wenn wir nun die acht Chromosomen welche aus dem Knäuel der Urpollenzelle sich herausgebildet haben, aus je zweier zusammengesetzt denken, und diese als *ab*, *cd*, *ef*, *gh*, *ij*, *kl*, *mn*, *op* bezeichnen, dann werden diese nach der ersten Alternative durch eine Längs- und eine Quertheilung die folgenden Vierergruppen bilden: $\frac{a^1|b^1}{a^2|b^2}$, $\frac{c^1|d^1}{c^2|d^2}$, $\frac{e^1|f^1}{e^2|f^2}$, $\frac{g^1|h^1}{g^2|h^2}$, $\frac{i^1|j^1}{i^2|j^2}$, $\frac{k^1|l^1}{k^2|l^2}$, $\frac{m^1|n^1}{m^2|n^2}$, $\frac{o^1|p^2}{o^2|p^2}$; und nach der zweiten: $\frac{a|b}{a|b}$, $\frac{c|d}{c|d}$, $\frac{e|f}{e|f}$, $\frac{g|h}{g|h}$, $\frac{i|j}{i|j}$, $\frac{k|l}{k|l}$, $\frac{m|n}{m|n}$, $\frac{o|p}{o|p}$. Und wenn wir zu diesen beiden die obengenannten Unregelmässigkeiten bei der ersten Theilung der Pollenmutterzelle in Betracht ziehen, dann werden die Variationen bei den gesammten Chromosomen noch grösser, als ich hier angegeben habe.

Tokyo, Ende Februar, 1897.

College of Agriculture,
Imperial University.

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Fig. 1. Epithelzelle von einer jungen Blüthenknospe in der Metakinese. Zupf-Präparat in Alcohol-Essigsäure mit Grenacher's Hæmatoxylin.

Fig. 2–5. Junge Urpollenzelle aus einer sehr kleinen ungestielten Antherenanlage. Flemming's Flüssigkeit mit Fuchsin-Methylenblau.

Fig. 6–8. Knäuelstadien ebensobehandelt wie die vorigen.

Fig. 9–10. Segmentation und Längstheilung der Chromosomen. Behandelt wie die obigen.

Fig. 11–17. Theilungsstadien der Urpollenzelle. Alcohol-Essigsäure mit Grenacher's Hæmatoxylin.

Fig. 18–20. Schlüsse der Theilung und Anaphasen. Ebensobehandelt wie die vorigen.

Fig. 21–23. Ruhende Zellen nach der Theilung—junge Pollenmutterzellen. Schnitte ebensobehandelt wie die vorigen.

Fig. 24–26. Vorbereitung zum Knäuelstadium. Schnitte mit Flemming's Flüssigkeit und mit Fuchsin-Methylenblau behandelt.

Fig. 27–28. „Synapsis"-Stadien. Schnitte ebensobehandelt wie die vorigen.

Fig. 29–35. Knäuelstadien. Man sieht in diesen Stadien einige Centrosomenähnliche Körperchen im Cytoplasma liegend, die wohl als solche gedeutet werden können. Fig. 29–32, Zupf-Präparat; 33–35, Schnitte. Fig. 29–30, Essigsäure-Carmin; Fig. 31–32, Alcohol-Essigsäure mit Grenacher's Hæmatoxylin; Fig. 33–35, Flemming's Flüssigkeit mit Fuchsin-Methylenblau.

Fig. 36–37. Segmentierung des Chromatinstrangs. Microsomen sind nun in zwei Reihen angeordnet. Flemming's Flüssigkeit mit Fuchsin-Methylenblau.

Fig. 38–44. Längsspaltung der Chromosomen. Fig. 38–42, ebensobehandelt wie die vorigen. Fig. 43–44, Essigsäure-Methylgrün.

Fig. 45–47. Längsgespaltene Chromosomen verkürzen sich stark, und haften sich an ihrer mittleren Portionen an einander, und so entstehen Figuren, die mit der in thierischen Genitalzellen bekannten Vierergruppe einige Aehnlichkeit haben. Flemming's Flüssigkeit mit Fuchsin-Methylenblau.

Fig. 48–70. Monasterstadien. Fig. 48–55, Alcohol-Essigsäure mit Grenacher's Hæmatoxylin; Fig. 56–61, Flemming's Flüssigkeit mit Fuchsin-Methylenblau; Fig. 62–70, Essigsäure-Carmin.

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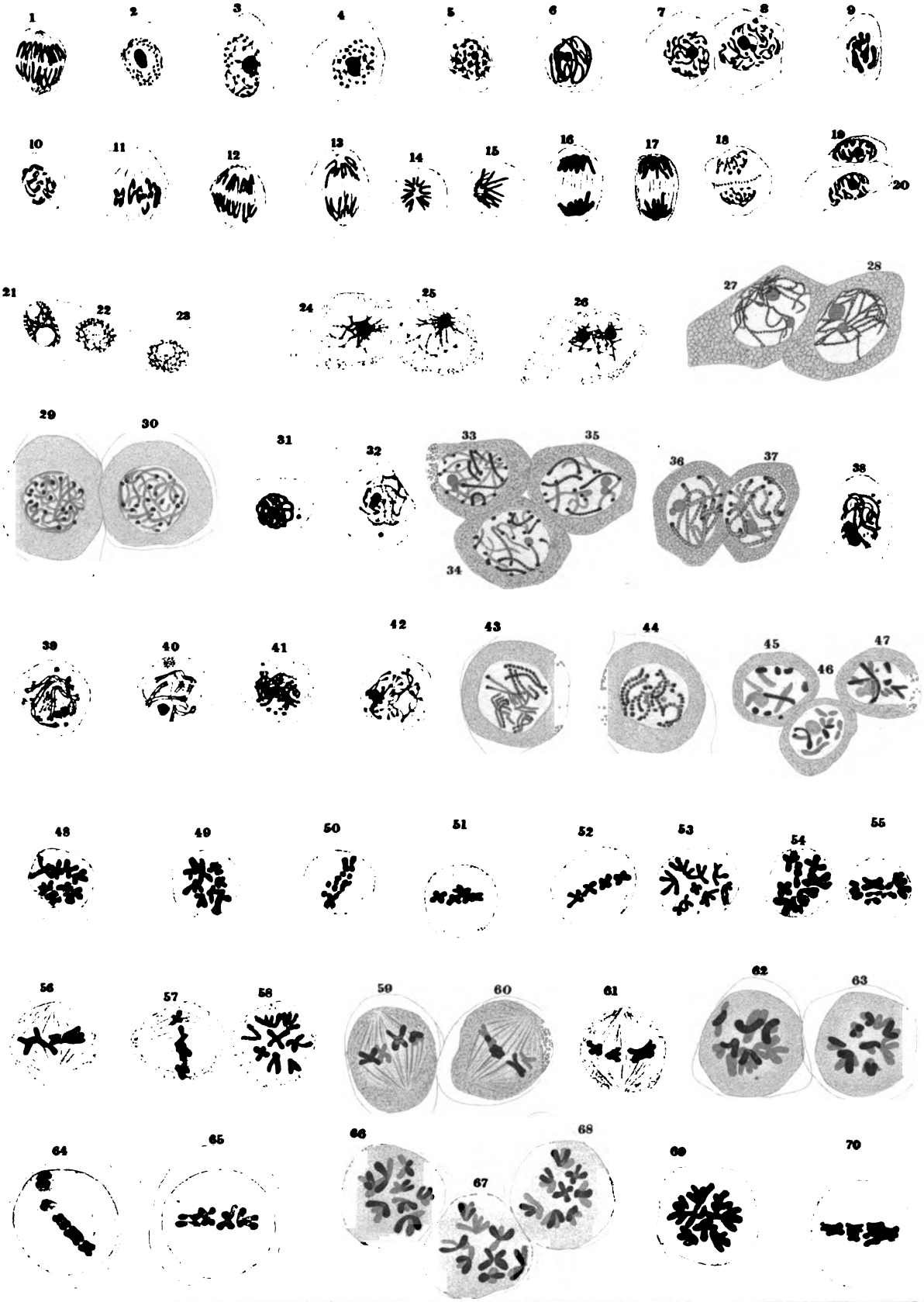

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Sämmtliche Figuren sind mit einem Zeichenapparat auf der Höhe des Objecttisches gezeichnet, und zwar mit Zeiss' Objectiv $\frac{1}{12}$ Homogener Immersion mit Ocular 4.

Fig. 71–77, 79–86. Dyastern und Metakinese. Man beachte in vielen Figuren (z. B. in Fig. 76, 83 u. 84), die Schenkel der V-förmigen getrennten Chromosomen an beiden Polen der Spindel sechszehn einfache Stäbchen entstehen. Fig. 71-77, behandlung mit vom Rath'scher Lösung mit Safranin-Gentiana-Orange Färbung; Fig. 79-86, Alcohol-Essigsäure mit Grenacher's Hæmatoxylin.

Fig. 78, 87–93. Entstehung der Zellplatte und Anaphasen. Die Pollenmutterzellen sind nun in Pollentochterzellen getheilt. Fig. 78, in vom Rath'scher Lösung, mit Fuchsin-Methylenblau; Fig. 87–93, Alcohol-Essigsäure mit Grenacher's Hæmatoxylin.

Fig. 94–104. Prophasen zur nächsten Theilung. Beide getrennte Schenkel von V scheinen sich nun wieder an ihren beiden Enden vereinigt zu haben. Fig. 94–102, mit Alcohol-Essigsäure mit Grenacher's Hæmatoxylin; Fig. 103–104, mit Essigsäure-Carmin.

Fig. 105-139. Mit Alcohol-Essigsäure mit Grenacher's Hæmatoxylin behandelt.

Fig. 105–107. Monasterstadien vor der nächsten Theilung.

Fig. 108–112. Dyastern.

Fig. 113–117. Ende der Metakinese und Bildung von vier Pollenzellen.

Fig. 118. Pollenzelle soeben befreit.

Fig. 119–121. Knäuelbildung.

Fig. 122–132. Längstheilung der Chromosomen bei der Vorbereitung zur Theilung von generativen und vegetativen Kernen.

Fig. 133–135. Dyastern.

Fig. 136–139. Anaphasen. Man beachte die Färbungsunterschiede zwischen den generativen und vegetativen Kernen.

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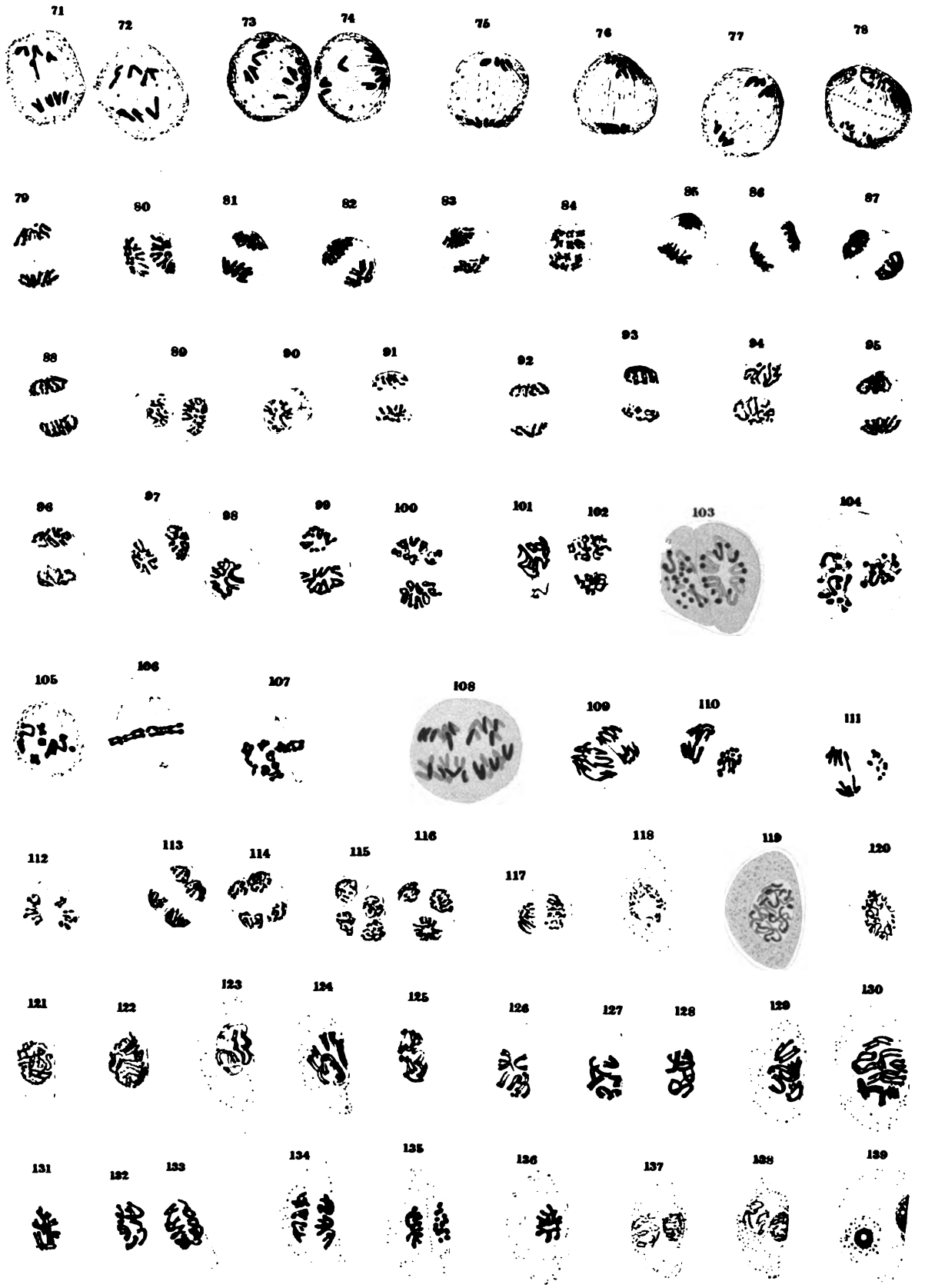

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Contributions to the Morphology of Cyclostomata

I. On the Formation of the Heart in Petromyzon.

by

S. Hatta.

With Plate XVIII.

In 1891, I published a paper entitled "On the Formation of the Germinal Layers in Petoromyzon" in this Journal, Vol. V. The present article on the formation of the heart is the first of a series, in which I propose to give the results of my study of later stages in the development of that interesting Vertebrate.

The materials and methods employed are quite the same as those given in the above-mentioned paper. An addition to the materials has, however, been made by a supply recently obtained at Kawagoye, a county-town not far from Tōkyō.

As the time passed through by the eggs and larvæ affords no measure of the progress of the development taking place in them, I give simple sketches (Fig. 1 A—H) showing the stages which I distinguish in the growth of the animal from the gastrula condition to that of the complete (free-swimming) larva.

Stage I. The gastrula:—The body has an ellipsoidal outline; the blastopore and the head-end are on the opposite poles of the ellipsoid. Fig. 1 A represents the oldest gastrula in which the head-fold has already become noticeable, being raised over the level of the blastoderm.

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STAGE II (Fig. 1 B) :—The head-fold is raised over the general level of the egg, and is protruded a little forwards (at the pole opposite the blastopore), although, being confined within the chorion, it is necessarily bent backwards and pressed down on the blastoderm. The neural ridge which was slightly marked in the foregoing stage becomes prominent along the dorsal longitudinal line.

STAGE III (Fig. 1 C) :—At first sight, this stage is very much like the preceding, but at the tip of the head-end, the dorsal median labial protuberance, and just ventral to it, the external mouth pit comes into view ; a few mesoblastic somites and two pairs (the first and the second) of the branchial invaginations can also be seen.

STAGE IV (Fig. 1 D and D') :—When the embryo of this stage is within the egg membrane, it is twisted in a peculiar manner, in consequence of the enormous prolongation of the head-fold, so that the head rests on the expanded yolk-sac ; three pairs of the gill-invaginations are visible. If the egg membrane is broken about the end of this stage, the embryo straightens and becomes retort-shaped (Fig. 1E.) It measures about 2.5 mm. and has from three to four pairs of the gill-invaginations. Corresponding with the external development, many profound internal changes take place, such as the first appearance of the pronephros, of the heart, of the liver, and so forth.

STAGE V (Fig. 1F) :—In this stage the embryo hatches spontaneously and is pistol-shaped ; it measures about 3 mm. with five pairs of the branchial invaginations.

STAGE VI (Fig. 1 G) :—The tail-end begins to grow and to point out, so that the yolk-expansion lies between the head-fold and the tail-protuberance ; it measures about 5 mm. and has from six to seven pairs of the gills. In this stage the gill-pouches begin to open outwards, and before it is over, all pairs of the persistent gills acquire free communication with the exterior.

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STAGE VII (Fig. 1H) :—The external features are almost the same as in the last stage, but the dorsal fin and a large part of the caudal are developed ; the stomodæum does not yet open externally, and the intestine is still crowded with the yolk-cells. The embryo is about 6.5 mm. long and has seven pairs of open gill-slits and one blind pair. One specimen had 73 pairs of the mesoblastic somites.

STAGE VIII :—The free swimming larva, having no longer any yolk-mass ; the intestine is filled up with diatoms. It is *ca.* 9 mm. in length and has about 97 pairs of the myotomes.

In Petromyzon, the heart is found, independent of age, in the same section of the body as the pronephros, the latter lying, in a lateral surface view (Fig. 1 H.), just dorsal to the former. Moreover, they are, during the earlier stage, of the same extent. The pronephros arises a little earlier than the heart. As these two structures appear just in the ventral aspect of the throat, where the slender head-fold is connected with the larger posterior portion, the study of them, in younger stages, is extremely difficult (see Figs. 3 and 5). Among the same series of sections, these structures are cut, in some frontally, in others transversely, and in still others obliquely. To ascertain facts concerning them, therefore, a number of series in various planes is necessary.

It will be well to begin this study with the stage when no trace of the heart is yet to be detected ; in such a stage is the embryo represented in Fig. 1C. In the angle which the crooked head-fold makes with the expanded posterior portion, the fore-gut is, from early stages (7 Plate XIV, Fig. 27), expanded into a wide space. The lateral plates of the mesoblast in this part grow downwards, from right and left, to meet in the ventral median line. A little before this meeting takes

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place, a number of cells quite like the mesoblastic elements, is found scattered between the epiblast and the hypoblast, being especially numerous on the median ventral line of that space; these are doubtless mesenchymatous cells.

Fig. 2 represents the section through the plane indicated by the line *a-a* in Fig. 1C; the cell-mass on the upper corner of the lateral plates on either side is the rudiment of the pronephric ducts and the second pair of the tubules (Fig. 2 *wd.*); the ventral free edges of the mesoblast do not yet meet and there is found no cellular elements in the median ventral line. The splanchnic plates consist of several layers of cells, while the somatic plate is only one cell thick. Fig. 3 shows a transverse section through the corresponding portion of an embryo a little older. The pronephros shows considerable progress; both the somatic and the splanchnic plates consist of a single layer of cells; the ventral free edges of the mesoblast do not yet meet. On the median ventral line a group of cells (*x*) not observed in Fig. 2 is visible. This is no other than the mesenchymatous elements referred to above. At the present stage, this mass of cells does not yet occupy a large extent, being traceable in only two or three sections. Figs. 4 and 5 are selected from a series of sections through an embryo a little older. Fig. 4 represents a transverse section through the slender neck portion just behind the second pair (the hindmost at this stage) of the branchial invaginations. In this, there is no trace of mesenchymatous cells in the ventral median line, but there are some disconnected cells on the ventral tip of the mesoblastic plates, falling in between the epiblast and the wall of the fore-gut. Fig. 5 is the fourth section behind Fig. 4, and, on account of the peculiar bend of the neck before referred to, is cut somewhat frontally (the plane of the section is shown by the line *a-b* in Fig. 1C). Moreover, as this is just at the angle which the slender neck portion makes with the larger posterior

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yolk-mass, the latter structure (lower portion of the figure) appears to be connected with the former (upper portion of the figure). The gut appears twice, viz. as the fore-gut in the neck portion and as the mid-gut in the yolk-mass. Some sections further dorsad, these two cavities will unite. The point to which I wish to call especial attention in Fig. 5 is the mass of mesenchymatous cells (*x*) lying between the fore-gut and the mid-gut. It is without any regular arrangement and is hedged in on each side by a very much thickened portion (*m.sp.*) of the splanchnic plates consisting of a row of high columnar cells. The thickening is limited to this small portion and ceases suddenly both anteriorly and posteriorly. It will be seen further on, that this portion plays an important rôle in the development of the heart, becoming, in fact, the pericardial wall.

By the rapid forward growth of the head-fold, the fore-gut is much elongated, but the mesenchymatous cell-mass, mentioned above, remains behind, lying next to that part of the yolk-mass which is subsequently transformed into the first section of the mid-gut. Figs. 6 and 7 are from a series of sections which passed somewhat obliquely in the plane indicated by the line *ab* (Fig. 1 D') through an embryo of about the same age as that figured in Fig. 1 D. The lateral plates of the mesoblast are thinned out everywhere, excepting that part which encloses the mesenchymatous cells. The latter are now arranged epithelially and already show a lumen in the anterior part ; this is the first trace of the ventricular endothelium, and the thickened portion of the splanchnic plates is, therefore, the pericardial coat.

Figs. 8, 9, and 10 are selected from the serial sections of the next advanced stage (Fig. 1 E). On the right side of Fig. 9 is seen the longitudinal section of the second pair of the pronephric tubules. The endothelium (*end*) encloses the lumen of the heart as a complete layer which is, in its turn, surrounded by the thick pericardial coat.

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The ventral free edges of the mesoblast almost meet in the ventral median line. Fig. 8 is the fifth section anterior to Fig. 9. The first pair of the pronephric tubules are sliced (*pn. t.*); here the mesenchymatous cells are still disjoined although the splanchnic plates embrace them from right and left (*x.* and *m. sp.*). The resemblance between this and Fig. 3 is very striking and we are, I believe, justified in concluding that the mass (*x.*) in Fig. 3 is destined to form the endothelium. Posteriorly to the section shown in Fig. 9, the mesenchymatous cells are either reticulated or aggregated, but are not epithelially arranged. Fig. 10 represents the third section posterior to that in Fig. 9; the mesenchymatous cells will be seen to be reticulated (*x.*).

The heart in this stage, therefore, shows already a tubular structure in its middle portion while it is only an aggregated mass of cells both anteriorly and posteriorly. It is enclosed right and left by the thickened splanchnic plates, and is suspended to the body-wall and the wall of the enteric canal.

Fig. 11 represents a transverse section through the portion corresponding to that in Fig. 9 of an embryo a little more advanced. The second pair of pronephric funnels are cut through (*pn. t.*); the endothelium is much thinned (*end*). The cardiac tube has now acquired a lumen through its whole length,——from the *truncus arteriosus* to the *sinus venosus*. It shows almost the same features everywhere. The dorsal and the ventral mesenteries or the *mesocardium posterius* and the *m. anterius* still suspend the heart in its entire length (*mc. p.* and *mc. a.*).

One step more (in the stage coinciding approximately with that represented in Fig. 1 G): these mesocardial suspensors begin to atrophy, the process beginning at the middle section and extending gradually both anteriorly and posteriorly, until finally the cardiac tube becomes

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free. I have endeavoured to show the process by the series of sections represented in Figs. 12, 13, and 14. In Fig. 13 the second pair of the pronephric tubules is cut through. The endothelial lining and the pericardial coat much resemble those in the former stage, but, by this time, the latter has lost all its connection with the pleuro-peritoneal membrane. The body cavity now ceases to be in separated chambers, its right and left halves uniting together (*p. p. c.*). This section passes through the ventricular portion of the heart. Fig. 12 represents the fourth section anterior to Fig. 13, and passes through between the first pair of the pronephric tubules and the posterior end of the branchial chamber. The splanchnic plates are comparatively thick at every point, and the *mesocardium posterius* still holds its place, while the *mesocardium anterius* has disappeared; this section corresponds with the *truncus arteriosus*. Fig. 14 represents the sixth section posterior to Fig. 13; the third pair of pronephric tubules are cut through; the funnels come into view in a bitaterally symmetrical manner. The heart is just as in the former stage: both the *mesocardium anterius* and the *m. posterius* (*mc. a.* and *mc. p.*) are present. The section is through the hindmost part of the heart, constituting the auricle and the *sinus venosus*.

Increasing in its length and breadth, the heart in the next older stage is constricted in the middle, being thus divided into conical halves, whose apices are turned respectively forwards and backwards. In this constriction the endothelial lining is of course involved, and thus comes to enclose two cavities connected together by a small aperture. The anterior chamber is a little larger than the posterior and constitutes the ventricle and the *truncus arteriosus* while the posterior forms the auricle and the *sinus venosus*. The aperture between them is no other than the auriculo-ventricular passage. Fig. 15 (from the stage corresponding with that of Fig. 1 H) passes through

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the heart, and being slightly oblique, shows the two cavities in it; in the next section after it the passage can be seen. In this stage all the mesocardial suspensorium atrophies and the cardiac tube is set free through its whole length.

In a little more advanced stage the pronephric tubules have become coiled many times and the ventral invagination of the liver has grown anteriorly and pushed aside the hinder part of the heart (Fig. 16).

From the foregoing observations it is clear that in Petromyzon the mesenchymatous cells, between the two primary layers at the point on the ventral median line where the head-fold joins the larger posterior yolk-mass is transformed into the endothelium of the heart. I am, therefore, unable to accept the statement of Shipley (18) that this is derived from the splitting of the splanchnic plates of the mesoblast. He says that (*loco. cit.* p. 17): "from the first appearance of the heart in the ventral mesentery its walls have been double; the splanchnopleure having split into two layers; of these the outer is at first much the thicker consisting of cubical cells; the inner layer is composed of comparatively flattened cells." It is probable that Shipley overlooked the earlier stages; for his Fig. 25, in which the heart is said to appear first, shows a stage already quite advanced corresponding with my Figs. 6 and 9.

The endothelium of the heart is stated by C. Hoffmann (9) and by J. Rückert (14) to arise from the hypoblast in Selachian embryos. C. Rabl (13) suggests the same in his account of Urodel Amphibia, while F. Schwink (17) thinks that it comes out, not from the hypoblast already formed, but from the yolk-mass, in both Urodela and Anura. J. Oellacher (12) derives it from the mesoblast in Batrachia. Paul Meyer (11) and F. Balfour (1) ascribe it to the same source in Selachia. A. Götte believes that it is in Petromyzon (6) a derivative of the

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hypoblast, and in Bombinator (5), of both the hypoblast and the mesoblast.[1]

As to the origin of the mass of cells, marked (*x*) in my Figs. 3, 5, 8, and 10, which is destined to become the endothelium, I am unfortunately unable to say anything definite, but I am inclined to regard it as derived from the mesoblast. These cells much resemble the mesoblastic elements: they are small, round, compact bodies, free from yolk-granules. Such a cell can not be detected in the hypoblastic layer, and there is found no trace of proliferation of cells in the ventral margin of the enteric wall, which presents a sharp contour throughout. On the contrary, the fact observed by both Shipley and myself that cells are detached from the free edges of the lateral plates of the mesoblast and fall into the space between the epiblast and the hypoblast, speak in favour of the view that the cell-mass may have been thus derived from the mesoblast.

Shipley believes that these detached cells are destined partly to form the ventral portion of the mesoblast and partly to give rise to the blood-corpuscles. Formerly I agreed with him in the first half of his statement, but further study forces me to conclude that these cells never have any share in the formation of the mesoblast, but are solely employed in building up the heart and the main trunks of the blood-vessels.[2] Their detachment and their accumulation in the ventral median line are observed both in the anterior and the posterior regions. Now there may be some reason in thinking that in the posterior region where the gastral mesoblast does not reach one half of the distance to the ventral median line, addition of free cells to its

[1] I have not read his complete work on Petromyzon: Abhandlungen zur Entwicklungsgeschichte der Thiere. 5. Hft. Die Entwicklungsgeschichte des Flussneunauges. Hamburg und Leipzig, 1888.

[2] It must be noticed that in the cardiac tube, when first formed, there is found no trace of blood-corpuscles.

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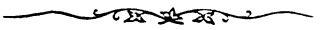
free edge may help its ventral growth, although I have never observed any thing which would justify such a conclusion. But in the anterior portion where the lateral plates of the invaginated gastral mesoblast early reach to, and meet, in the ventral median line, there is no need of free cells to be added to the ventral edges to help their growth. As far as my observations go, these cells are all used in building up the vascular system. It seems most probable that the lateral and ventral parts of the mesoblast are formed solely by the downward growth of the dorsal part; for many mitotic figures are, even in much later stages, seen in the mesoblast, so that it needs no contribution from the free cells in question.

From its first appearance, the heart is a single endothelial tube; there is no reason to think it has a paired origin.

My deepest thanks are due to my esteemed teacher, Prof. K. Mitsukuri, for his kindness in looking through the manuscript of this paper.

Biological Laboratory, the Gakushuin (Nobles' School), April 1897.



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Explanation of Figures.

Complete list of reference letters.

a. anus.
ao. aorta.
a.v. auriculo-ventricular septum.
bp. blastopore.
br.i. branchial invagination.
br.c. branchial cleft.
ch. chorda dorsalis.
e.m.p. external mouth-pit.
end. endocardium.
ep. epiblast.
f.g. fore-gut.
h. heart.
hy. hypoblast.
l.m. lateral plates of mesoblast.
l.p. labial protuberance.
m. mouth.
mc.a. mesocardium anterius.
mc.p. mesocardium posterius.
m.g. mid-gut.
m.gp. splanchnic plate of mesoblast.
n. neural cord.
p.c. pericardium.
pn.t. pronephric tubule.
p.p.c. pleuro-peritoneal cavity.
p.p.m. pleuro-peritoneal membrane.
s.ch. subchorda.
s.m. somatic mesoblast.
t.a. truncus arteriosus.
w.d. Wolffian duct.
x. mesenchymatous cells.
ye. yolk-cells.

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PLATE XVIII.

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Plate XVIII.

Fig. 1.—A—H are representations of successive stages of the embryo. A,B,C,D, and D′ are magnified about 28 diameters; E, F, G, and H about 13 diameters. D′ is a view of the other side of D.

Fig. 2.—Transverse section through the neck of the head-fold of an embryo at about the same stage as that represented in Fig. 1C. Zeiss C × 2.

Fig. 3.—Transverse section from an embryo of a little more advanced stage; the section has passed through almost the same region as that of Fig. 2. C × 2.

Figs. 4 and 5.—Series of transverse sections from an embryo more advanced than that of the last Figure. Fig. 4 is the fourth section anterior to Fig. 5; the former section is cut transversely, while the latter has passed through somewhat to the front. C × 2.

Figs. 6 and 7.—Series of transverse sections through a still more advanced embryo; the section has passed through an oblique plane indicated by the line a b in Fig. 1D′. Fig. 7 is the section next posterior to Fig. 6 and has been somewhat reconstructed from the section that follows, to make the relative position of the germinal layers intelligible. C × 2.

Figs. 8, 9, and 10.—These have been selected from a series of transverse sections through the neck of the head-fold of an embryo of about the same stage as that of Fig. 1 E. Fig. 8 is the foremost section of the three; Fig. 9 is the fifth, and Fig. 10 the eighth, section after Fig. 8. C × 2.

Fig. 11.—Transverse section from the embryo of the same age as that of Fig. 1E. The section has passed through the same region as in Fig. 9. C × 2.

Figs. 12, 13, and 14.—Transverse sections from the neck of the head-fold of a little more advanced embryo. Fig. 12 is the most anterior; Fig. 13 is the fourth and Fig. 14 the eleventh after Fig. 12. C × 2.

Fig. 15.—Transverse section through the neck of the head-fold of an embryo of the same age as that represented Fig. 16. C × 2.

Fig. 16.—Transverse section through the neck of the head-fold of an embryo of about the same stage as that in Fig. 1H. C × 2.

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Vol. X Pt. 2 . . . *yen* 1.20.

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明治三十年六月廿三日印刷
明治三十年六月廿五日發行

編纂兼發行者　帝國大學

東京市日本橋區兜町貳番地
印刷者　星野錫
東京印刷株式會社

東京市日本橋區通三丁目
十四番地
賣捌所　丸善株式會社書店

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東京帝國大學紀要

理科

第十冊第三號

THE

JOURNAL

OF THE

COLLEGE OF SCIENCE,

IMPERIAL UNIVERSITY OF TOKYO,

JAPAN.

VOL. X, PART III.

東京帝國大學印行

PUBLISHED BY THE UNIVERSITY.

TŌKYŌ, JAPAN.

1898.

MEIJI XXXI.

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The Metamorphosis of Asterias pallida, with Special Reference to the Fate of the Body Cavities.[1]

by

Seitaro Goto.

Professor in the First High School, Tōkyō.

With Plates XIX—XXIV.

This paper embodies a full account of the results of my work on the metamorphosis of *Asterias pallida*, of which a preliminary report has been published in German ['96 *a*] and in English ['96 *b*]. To Dr. A. Agassiz I am indebted for his courtesy in enabling me to spend several weeks in his Laboratory at Newport, R. I., and to Prof. E. L. Mark, under whose direction the work was done, I am obliged for his friendly interest in my work and valuable suggestions; and it gives me much pleasure to express my best thanks to both of these gentlemen.

The material was fixed in the following mixture:

Saturated solution of corrosive sublimate .....80 cc.
Concentrated glycerine .....15 "
Glacial acetic acid ..... 5 "

The larvæ were either dropped from a pipette into the mixture or else they were put into a watch glass with some water, the water then softly drawn out with a pipette until just enough was left

1) Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of E. L. Mark, No. LXXXVIII.

To avoid misunderstanding the writer begs to state that owing to geographical separation it has not been possible to submit the manuscript or proof sheets of this paper to Prof. Mark before publication.

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to allow the larvæ to float, and the mixture then poured in. The larvæ were left in it for 3–5 minutes, then passed through the ascending grades of alcohol, beginning with about 30%, and finally preserved in 70% alcohol, in which the remaining sublimate was thoroughly washed out with iodine. The result was very satisfactory, except that, as I had noticed while in Newport, the volume of the larvæ was perceptibly diminished, when they were left too long in the mixture, evidently owing to osmotic pressure. To prevent this when I was working with the eggs of the sea-urchin last winter I diluted the above mixture with distilled water to the specific gravity of sea-water, viz. to $2^1/_2$ times the original volume roughly. This diluted mixture I can recommend as excellent for many delicate pelagic larvæ (annelid larvæ, mitraria, ephyra, small hydromedusæ, etc.).

The general features of the development of the present species are well known from the excellent paper of A. Agassiz ['77]; I have therefore only drawn, in Plate XIX, the principal stages that come into consideration in this paper, beginning with a fully developed bipinnaria and reaching up to a young star, in which the permanent mouth is being developed. These stages, seven in all, will be referred to in the order of their age by the letters of the alphabet (Pl. XIX).

I. General Form of the Body.

Externally the bipinnaria is distinguished by its perfect bilateral symmetry, and no one has, so far as I know, succeeded in establishing a genetic relation between the principal plane of this bilateral symmetry and the axis of the radial symmetry that preponderates later. For example, one of the latest students of echinoderm morphology has observed that "le plan de symétrie bilatérale acquis secondairement ou déterminé par l'existence d'organes impairs, n'a aucun rapport avec le plan de

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symétrie de la larve et il est tout à fait superflu, au moius dans l'état actuel de nos connaissances, de se baser sur ce dernier pour déterminer le premier" [Cuénot, '91, p. 331]. And yet, if I am not greatly mistaken, I believe that I can prove a direct connection between the two principal planes in question; and I may state at the outset that this connection is that of exact coincidence.

As an important preliminary step to the discussion of this question, we must make our mind clear as to the method by which we are to determine the plane of reference. This is indispensable, as nearly all externally visible organs shift their positions considerably in the transition from the larval to the adult form. It is therefore essential that we should take hold of some organs, the relation of which to the plane of the larva is clearly known and which persist until the aboral disc has assumed its final position. Such organs of reference are found in the three brachiolar arms. One of these arms is dorsal and median, and the other two are ventral and paired, so that the sagittal plane passes through the tip of the dorsal median arm and makes a right angle with the line that connects the tips of the two paired arms. A glance at Plate XIX will show that these brachiolar arms persist up to a stage in which the adult form is all but ready, keeping all the while, their relative positions. I have taken them as my landmark for two reasons: (1) they are almost the only externally visible organs that are common to both the larva and the adult form; (2) the plane determined by them cuts the body in two equal portions, passing through the mouth and anus while these are present, and, after their disappearance, through the places where they were.

As already known from the works of preceding students, the aboral disc (a convenient term for the ectodermal thickening that is to form the aboral side of the star) is formed on the right side of the larva in the posterior portion of the body (Pl. XX, Fig. 17). It is

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irregularly curved, but if, for simplicity's sake, we regard it as a plane disc, it makes, when first formed, an angle of about 30° with the sagittal plane of the larva. As metamorphosis goes on, however, it gradually rotates counter-clockwise[1], so as to make a greater angle with the same plane. This rotation continues until the aboral disc comes finally to be at a right angle to the sagittal plane. These statements are abundantly proved by Figs. 13-14, Pl. XIX and Figs. 17 & 18, Pl. XX, as well as by many sections which are not reproduced here.

The primary water-pore when first formed is on the left side, but later it is found in the median line or even slightly but distinctly on the right side (Fig. 19, Pl. XXI)—a position which it keeps permanently. Now, from the above description it will be clear that the sagittal plane of the larva cuts the disc of the star at right angles and passes through the water-pore and the centre of the disc, that is to say, the sagittal plane of the larva and the plane of bilateral symmetry of the star are coincident. The arms of the starfish may therefore be justly spoken of as the median ventral, the right and left dorsal, and the right and left ventral, arms. It need hardly be added that the oral side is anterior, and the aboral side posterior.

The rotation of the aboral disc above described will perhaps be more clearly understood and can be graphically represented by making two arbitrary suppositions, which although not accurately realised in nature, still are, in my opinion, perfectly allowable in the interest of clear exposition, viz., that the aboral disc is a perfect circle and that one of its diameters is parallel to both the sagittal and transverse planes. If then we project this circular disc by the usual method on the sagittal and transverse planes we shall get a circle or ellipses of different

1). In describing the rotation of planes we shall suppose, unless otherwise stated, that the observer is looking from the ventral side of the larva.

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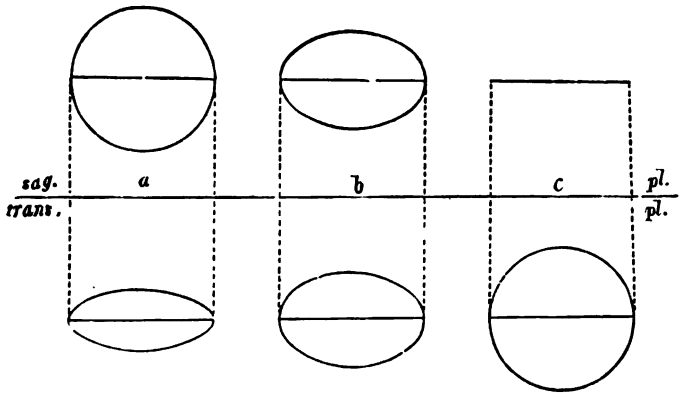
eccentricities, according as the disc is parallel or more or less inclined, to the plane of projection. In the accompanying cut the sagittal plane is represented by the portion above the horizontal line and the transverse plane by the portion below it. As stated above, the aboral disc, when it distinctly appears for the first time, makes an angle of about 30° with the sagittal plane, and therefore we get on the sagittal plane an ellipse of comparatively small, and on the transverse plane one of great, eccentricity (*a*); when the disc makes an angle of 45° with both planes we get ellipses of equal eccentricities (*b*) and when the disc assumes its final position its projection on the sagittal plane is a simple straight line, while on the transverse plane it comes out as a circle. We thus get different *phases*, as one may express it, of the aboral disc according to its varying positions with respect to the two principal planes of reference.



Cut 1.

We shall now examine the assumptions, on which the above graphic method has been developed. The first, that the aboral disc is a perfect circle, needs hardly any discussion. We may remark that the ectodermal thickening which gives rise to the aboral side of the star has always a form more or less approaching a flat circular dome. As to the second assumption, that one of the diameters of the aboral disc, imagined as a perfect circle, is parallel to the intersection of the sagittal and transverse planes, we must say that as a

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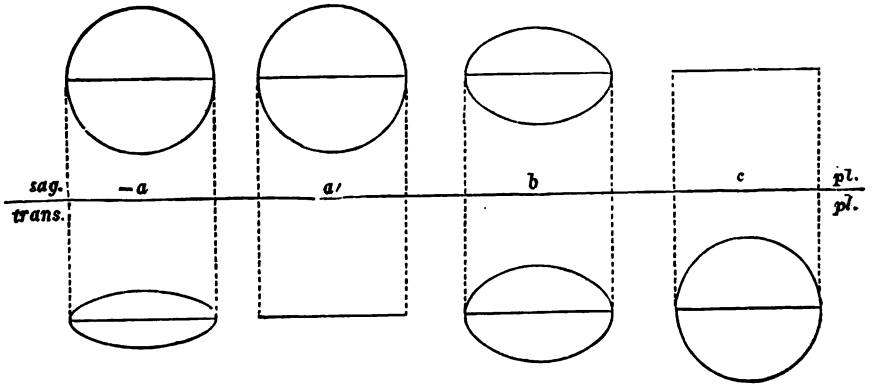
matter of fact the aboral disc is inclined to both these planes, and consequently that the major axis of the projection ellipse will not be equal to the diameter of the aboral disc, as it is supposed to be in the cut, except in its final stage (*c*). With this correction, however, the above description is accurate.

Hand in hand with the rotation of the aboral disc, the oral disc (this time an entirely imaginary disc, whose plane passes through the rudiments of the water-vascular system) also rotates in the same direction, but the total amount of its rotation is greater than that of the aboral disc. This follows from the fact that the oral disc in its initial position makes a negative angle with the sagittal plane, while the aboral disc makes a positive angle with it; an angle being termed positive when its position is arrived at by turning a plane counter-clockwise, and negative when it is attained by turning a plane clock-



Cut 2.

wise, and 90° being considered the limit of rotation in either case. So if we draw projection figures of the oral disc in the same way as we have done with the aboral disc, we shall get more phases than in the case of the latter, as shown in cut 2. In this cut the corresponding phases are marked by the same letters of the alphabet as in cut 1, but with a negative sign when the oral disc in corresponding stages makes

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a negative angle with the sagittal plane, and *a'* being evidently the phase when the oral disc is parallel to the sagittal plane. Phase *a* is simply omitted.

The correction needed for the aboral disc is entirely superfluous in the case of the oral disc, as one of its diameters is always practically parallel with the intersection of the sagittal and transverse planes.

The rotation of the disc above described has been observed in a bipinnaria by Metschnickoff ['69, p. 36 *et fort*]; in *Asterina gibbosa* by MacBride ['96, p. 355], who enumerates it as one of the characteristic features of its development; and is also well shown in one of Ludwig's figures ['82, Pl. VI, Fig. 80=MacBride '96, Pl. XVIII, Fig. 14]; but its significance has evidently escaped the attention of the latter, and MacBride has drawn, it seems to me, an entirely mistaken inference. He says, "The adult plane makes an angle of about 70° or more with the larval plane; but without any very serious error, it may be regarded, for purposes of description, as at right angles to it: thus the direction right to left, according to the larval plane, becomes aboral to oral according to the adult plane, and dorsal to ventral according to the larval plane is nearly parallel to the adult plane" ['96, p. 357]. By larval plane MacBride means the frontal plane of the larva. But the adult plane makes a right angle not only with the frontal, but also with the sagittal, plane of the larva; therefore the direction right to left *as well as* dorsal to ventral in the larva is parallel to the adult plane. Bury ['95, p. 70] also assumes that in *Bipinnaria asterigera* the sagittal plane of the larva and the plane of the star coincide; but in view of the facts brought out in the above discussion, I think I may say that this will also be shown, on further study, not to be the case, but that the two planes make a right angle with each other. Again, if the orientation of the body above explained is correct, then the aboral pole of the asterid and that of the crinoid are, as justly

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observed by MacBride, *not* homologous, and the arrangement of calcareous plates in the two classes must be looked upon as simply a remarkable case of homoplasy. To still maintain the homology of the poles of the same name in the asterid and crinoid would involve an assertion of the homology of the posterior pole of the crinoid larva, with the anterior pole of that of the asterid—an assertion which, for all that we know, would be too arbitrary and too much against facts.

The foregoing description has been kept for clearness' sake somewhat abstract. Let us now see the process exactly as it occurs in nature. In stage B the median portion of the ectoderm lying dorsal to the stomach is considerably thickened (Figs. 21 & 22, Pl. XX), the cells being highest in the median line and thence decreasing towards either side. The aboral disc is formed by the extension of this thickening towards the right side of the body. In stage C the thickening has extended, posterior to the anus, in many specimens to near the ventral median line (Fig. 17, Pl. XX); but this is not always the case, for, as we see from Figs. 19 & 20, Pl. XX, which are taken from a specimen in stage C, the thickening is still confined to the dorsal median portion of the body. In stage D, however, the thickening has passed the ventral median line and has extended so far anteriad that its antero-ventral margin overhangs the protoproct. In the posterior end of the body also it has encroached on to the right side. From this stage onwards, the aboral disc seems to grow, to a large extent though not solely, by a change of the surrounding cells from a flat to a cylindrical form; for, while mitotic figures are rarely, if at all, observed in the ectoderm, the præoral portion, and especially that portion of the body lying between the protostome and the aboral disc, steadily decreases in size, until, passing through stages E and F, it finally reaches stage G, in which the portion adjoining the mouth has been already incorporated into the oral disc; and the præoral lobe hangs down as a mere appendage

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from near the centre of the oral side. In stage E the aboral disc is inclined at an angle of about 45° to the sagittal plane, and in F it is at right angles to it. It has here assumed its final position and from now onward the præoral lobe has only to shrink in for the star to be complete. It may be stated in addition that the præoral lobe is, just before its final disappearance, situated on the right side of the oral disc.

As stated above, the aboral disc grows, in its later stages, largely by a change of form of the cells of the surrounding region. This means that the shrinkage of the præoral lobe and the portion adjoining the mouth is due to the same cause. So far as I have observed, the thickened patches between the arms of the brachiolaria and their thickened tips are the only parts that undergo histolysis. While the general ectoderm stains blue with hæmatoxylin, these patches show, after stage F, a decidedly reddish tinge, and numerous amœbocytes are observed in their vicinity.

As to the nature of the thickened patches between the brachiolar arms I can not say anything positive. In *Asterina gibbosa* MacBride has observed in a corresponding position a glandular patch, by which the larva attaches itself very effectively to external objects while it is undergoing metamorphosis; Bury also states that "metamorphosis is ushered in, in the larva of *Asterias rubens*, by the fixation of the larva by the knobbed arms of the brachiolaria, and the ciliated pit which they surround" ['95, p. 74]. In view of these facts as well as of the general appearance of these patches which look suspiciously glandular, I have tried Bismarck brown; but whether from some fault in the brown[1] I used, or owing to a real absence of glandular cells, I have not succeeded in obtaining a differential stain. The latter alternative appears to me more probable, as the brachiolaria or transforming larva

1). It is stated to have come from J. D. Riedel, Berlin.

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is wholly unable to attach itself to any object, but helplessly floats about until it has completed its metamorphosis, when it can attach itself very effectively to any substratum, by means of the ambulacral feet which are developed, a few pairs in each radius, by this time. It is also to be remarked that the histolysis of the thickened patches is all but completed in stage G, when the larva is still wholly unable to fix itself to any object.

The degeneration of the thickened patches of the brachiolaria arms brings us to another problem, viz. the function of amœbocytes. I have stated above that these wandering cells are crowded in large numbers around the parts that are undergoing histolysis. We must not, however, take this fact in itself as an evidence of the phagocyte action of these cells. It may be a case of simple chemotaxis, the amœbocytes being positively tactic towards some acid or acids that are generated during histolysis. In fact, so far as I could observe with the highest magnifying power at my disposal (Zeiss oil imm. $^1/_{12}$) I could not demonstrate the presence of any solid particle in these cells; it has seemed to me that they assimilate the liquid substances produced by histolysis. In the transforming stages, i.e., between the almost complete disappearance of the præoral lobe and the formation of the definitive mouth, innumerable amœbocytes are found in and among the cells of the gut.

At the end of this section treating of the general form of the body, a few words may be added with regard to the behaviour of the general ectoderm during metamorphosis. It has already been stated that the disappearance of the præoral lobe is largely due to a gradual thickening of its ectoderm. The same process of thickening takes place all over the body; the tentacles are gradually drawn in, and as these are provided with a thick ectoderm they enter directly into the formation of the oral disc. In doing so, however, they

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undergo an additional thickening, and in some sections I have observed indications of the intimate connection of their ciliary band with the nervous system of the star. As a necessary consequence of this thickening of the ectoderm, which takes place all over the body, the star which has just been formed is considerably smaller than the larva, as may be seen at a glance on Pl. XIX.

II. Gut.

It was thought by Agassiz ['77] that the larval mouth and anus persist. My observations, however, have led to a different result: both the larval mouth and anus atrophy. In the following account I shall therefore, adopting with trifling modifications, the terminology of Roule [Embryologie comparée], speak of the *protostome* and *metastome* and the *protoproct* and *metaproct*.

Protostome.—The protostome atrophies by a constriction of the œsophagus and subsequent evagination of the portion which is connected with the ectoderm; but the exact stage and the precise point at which this takes place seem to vary somewhat. As to the second point it would be natural to expect that just so much of the œsophagus would be evaginated as was invaginated during the formation of the protostome, thus assigning the ectoderm and endoderm to their proper spheres in the adult body; and I believe this is exactly what takes place in nature. But at the same time we must admit that there are variations from this rule, as will be seen from the description which follows.

In stage E the œsophagus presents numerous irregular constrictions (Fig. 10, Pl. XIX), and its final division is effected at one of these constrictions lying near the mouth, some time between stages F and G. In one series of sagittal sections of a specimen but slightly more advanced than stage F, however, the division was effected at the

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junction of the œsophagus and stomach. I could observe nothing that suggested any abnormality in this specimen, nor anything to show that this division was due to mechanical injury. It seems to me therefore that we must admit the occurrence, in some cases, presumably few, of such a variation from the rule. There is nothing that requires any special description connected with the atrophy of the protostome; the portion connected with the mouth, that was cut off from the rest being again evaginated and forming a portion of the oral disc, while the portion connected with the stomach becomes thicker and shorter and forms the definitive œsophagus. During this change the cardiac valve of the stomach becomes less distinct and finally disappears altogether (cf. among others, Figs. 9, 10, Pl. XIX, & Fig. 39, Pl. XXIII). The metastome is formed by the coalescence of the œsophagus with the ectoderm of the oral side and its subsequent breaking through. This takes place in stage H (Fig. 16), and is represented in section in Fig. 36.

Protoproct.—During the passage from a fully developed brachiolaria to the transforming stage the larval anus is considerably shifted to the right side of the body, and in a stage intermediate between stages F and G, it lies just beneath the aboral disc between arms I and II, nearer the latter (Fig. 40, Pl. XXIII). [For the numbering of the arms see figures on Pl. XIX.] It begins to atrophy at this stage, the process being inaugurated by a fusion of the ectoderm forming the rim of the anus (Fig. 32, Pl. XXII). The portion of the intestine adjoining the anus is then partly constricted off from the rest, and subsequently undergoes histolysis. In Fig. 32 one can see this process going on; and the character of the nuclei of the epithelium, the presence of numerous amœbocytes and of a finely granular substance in the vicinity, and the general indistinctness of the surfaces of the epithelium all prove that this portion is actively undergoing disintegration.

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The portion of the intestine that remains connected with the stomach is taken in in the formation of the stomach of the adult.

Permanent gut.—From the above description it is clear that the larger part of the larval gut enters into the formation of the permanent gut. The intestine hangs on to the stomach for a while but little changed in form, but in stage H it can not be recognised as such. The œsophagus, however, is never completely incorporated with the stomach but always projects more or less orad. In stage E (Fig. 10) the stomach is already seen to send out indistinct diverticula into the rudiments of the arms. These diverticula grow into the arms as these are developed; they are at first simple, but in a star whose radius is 2.5 mm. they are distinctly double. In this young star the rectal vesicle as well as the metaproct is already developed. The former is at this stage an irregularly ovoidal sac situated in the right dorsal interradius, but extending more into the base of the right ventral arm, and is connected with the stomach by a short but distinct canal. Its aboral wall is directly applied to the aboral disc, so that no free space is left between the two. We shall afterwards see how this affects the schematic arrangement of the body cavities. From the rectal vesicle proceeds obliquely aborad a short rectum, which opens in the same interradius as that in which the vesicle is situated, but near the centre of the disc, as made out by Ludwig long ago ['80].

I call œsophagus that portion of the definitive gut which can be evaginated outside the body. This portion is composed of cells which differ in character from those of the rest, these cells being slender and cylindrical and closely set and with nucleus elongated oval, while in the remaining portion of the gut, the hepatic cœca included, the cells are mostly cubical and the nucleus spherical. It is perhaps needless to remark that the hepatic cœca are connected with the aboral end of the gut.

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III. Enterocœl and Hydrocœl.

From their genetic connection as well as from their undergoing simultaneous changes, it will be most convenient to treat these two systems together.

The general aspect of the changes which these two systems undergo is shown in Plates XIX–XXI, and we shall use them as basis in the following description. I start with stage B, i. e., a fully developed bipinnaria, since the earlier stages have, in my opinion, been satisfactorily studied by previous observers. The hydro-enterocœl at the stage just mentioned is represented in Figs. 2–4, in its left, ventral, and right aspects. As will be seen from these figures there is but one cavity, but we may distinguish different portions of it. Since we know that the cavities on the two sides of the body were, after their separation from the primitive gut, entirely separate and united secondarily in the præoral lobe we shall distinguish them as the right and left enterocœls. In either of them we may again distinguish two portions, the anterior and posterior. In the left enterocœl these two portions are pretty sharply marked off by the presence of the pore-canal and a sudden constriction which the cavity undergoes at this point, due apparently to the development of the hydrocœl. In the right enterocœl, however, these two portions grade insensibly into each other, although in an earlier stage a slight constriction can often be observed at the corresponding level. At this stage, therefore, we may already distinguish four portions, viz., the right and left anterior and the right and left posterior, and we shall subsequently see that their separation becomes more and more accentuated as metamorphosis progresses.

We must now take a more careful view of the several portions of the body cavity. The two anterior enterocœls freely communicate

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with each other in the præoral lobe and send out from their anterior extremity three diverticula into the rudiments of the brachiolar arms, which are just visible as out-pocketings of the body-wall. The posterior extremity of the left anterior enterocœl is directly continuous with the rudiment of the aquiferous system; indeed there is no natural boundary between the two at this stage, and we can only draw an arbitrary line on the basis of subsequent results. The pore-canal, which, be it remarked at the outset, I look upon as belonging to the enterocœl and not to the hydrocœl, as will become clear at a later stage of our description, is very short and opens into the postero-dorsal corner of the left anterior enterocœl. Its external opening is on the right side of the median line. The left posterior enterocœl is U-shaped in both its lateral and ventral aspects, being most spacious at the posterior end of the body. The ventral horn of the U as seen from the left side extends just posterior to the anus and here curves backwards on the right side and ends blindly a little before reaching the posterior end of the body, its wall being closely appressed to that of the right posterior enterocœl. The latter looks somewhat spacious in its lateral aspect but is considerably compressed from side to side. It does not, moreover, extend very much ventrad, owing to the encroachment of the left posterior enterocœl on the right side. Excepting in the præoral lobe, the cavities of the two sides of the body are separated by a mesentery on the whole of the dorsal, and the greater part of the ventral, median line. At the posterior portion where the left cavity extends to the right side, the mesentery is removed to the right to a corresponding extent.

The rudiment of the hydrocœl is present in the form of five out-pocketings of the posterior portion of the left anterior enterocœl, and of a central cavity enclosed by connecting the bases of these diverticula. These out-pocketings are, it is needless to say, the rudiments of the

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radial water-tubes, and their walls are considerably thickened. I number them as marked in the figures, i. e., I begin on the ventral side and proceed counter-clockwise[1], as seen from the left side. No. 2 is the first developed and is situated hindmost, 1 and 3 are formed almost simultaneously, then 4, and lastly 5. The latter is situated anteriorly to the opening of the pore-canal into the enterocœl and is somewhat removed from the dorsal margin of the left enterocœl.

In *Bipinnaria asterigera* Bury ['95, p. 66] has observed that the ventral horn of the left posterior cavity curves over to the right side and fuses with the right body-cavity; and he adds, "This fusion makes it impossible to determine the exact limit of the two cavities (right and left), but we shall probably not fall into any important error if we regard the point of junction as median." In *Asterias pallida* this fusion is effected in the next stage and the line of fusion is, as can already be inferred from the foregoing description, wholly on the right side of the body.

It remains to speak of the "dorsal sac"[2] observed by previous students in this stage [Field '93, Bury '87 & '95, MacBride '96]. Field and Bury agree in regarding it as of mesenchymatous origin; MacBride, on the other hand, has advanced an entirely different opinion. According to him it represents the right hydrocœl and is budded off from the right posterior extremity of the anterior cœlom, with which it is, for some time, connected by a string of cells; this string of cells, however, soon atrophies and the cavity in question is then left as a closed vesicle in the midst of the mesenchyme ['96, p. 349]. Further in MacBride's opinion "the right hydrocœl persists

1). This is the same method of numbering as that used by Ludwig. MacBride, on the other hand, begins at the other end and proceeds clockwise.

2). Called by Bury in his paper of '87 "blood vascular space." "Dorsal sac" is the name employed by him in his later paper ['95].

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in the adult as a closed sac just under the madreporite, and has been seen here by Cuénot....."

The vesicle in question is present, in *Asterias pallida*, in stage B and also prior to it. Fig. 21, Pl. XXI, represents a cross section at about the level of the water-pore, of a specimen in a stage a little earlier than stage B. Here the sac is situated in the mid-dorsal line, and is somewhat flattened. It runs usually through only three or four sections (each=6–7 μ) and is connected by a somewhat scattered string of cells with the *left* enterocœl, just behind the internal opening of the pore-canal, that is to say at the boundary between the anterior and posterior enterocœls. In later stages it can be recognized as an extension of the left anterior enterocœl on the dorsal side of the stomach, and in the adult it is incorporated with the axial sinus. The result of my observation is thus different from that of MacBride, and for the sake of completeness I may add that the closed sac observed by Cuénot ['87, p. 77) directly under the madreporite is constricted off from the axial sinus later, as will be described further on. With regard to MacBride's observation above cited it must be remarked that the string of cells connected with the vesicle in question extends very often close to the dorsal margin of the right enterocœl, but I have satisfied myself that it arises from the left body-cavity.

We shall now pass on to stage C (Figs. 5, 6, 7, & 8, Pl. XX). I shall first describe the different cavities and then explain how the transition from the previous stage to this is effected. In the præoral lobe there is nothing special to mention except that the brachiolar arms have become tolerably well developed and with them the diverticula from the anterior enterocœl have considerably enlarged. The left posterior enterocœl has grown dorsad, and on the ventral side it has also extended considerably over to the right side of the body, and forms one large cavity around the anus, the primary mesentery between

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it and the right enterocœl having disappeared altogether (Fig. 7, Pl. XIX, Fig. 17 *a* & *b*, Pl. XX). There is, however, an incomplete valve-like septum at the posterior end of this common cavity in about the same position as the boundary of the right posterior enterocœl in stage B. Again, looking at the larva from the right side (Fig. 8, Pl. XIX), we see that a portion of the right posterior enterocœl has been cut off from the rest at the dorsal posterior corner of the body, forming here a crescent-shaped closed cavity extending from a little in front of the water-pore to the posterior end of the body. From the position which it occupies in the complete star I shall call it "epigastric enterocœl." The mesentery lying between it and the rest of the body cavity on the right side has no genetic connection whatever with the primary mesentery separating the right from the left posterior enterocœl, as will be clear from the following description explaining its formation.

The very beginning of the septum in question can already be observed in stage B. Fig. 21, Pl. XXI, represents a portion of a transverse section of a specimen in such a stage, some seven sections (each= 6–7 μ) behind the water-pore. The left posterior enterocœl extends here over the mid-dorsal line—a result of an active proliferation of the cells of its dextro-dorsal wall, with which the formation of the "dorsal sac" is connected. In a somewhat dorsal portion of the right enterocœl one sees a somewhat indistinct septum formed by the coalescence of the epithelium. It runs through only a few sections, and at places there is no septum, but the walls of the two sides are simply closely appressed to each other. The latter condition persists to a rather late stage in the posterior portion of the body, although in the more anterior part, *i.e.*, near the water-pore, the mesentery (using the term with its usual connotation, *i.e.*, two layers of epithelium with mesenchyme between) is completed. Compare Figs. 23 & 24, Pl. XXI, the latter of which is three sections behind the level at which, in this particular

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-case, the mesentery is complete. In Fig. 22, Pl. XXI, I have reproduced a section taken from a stage slightly prior to stage C, in which there is an additonal septum beside the mesentery separating the epigastric from the right posterior enterocœl ; showing that there is in this region a somewhat accentuated tendency to form septa. The mesentery in questionis completed some time after stage D (Figs. 17, Pl. XXI).

The transitional steps by which the condition of the body cavities in stage B passes into that in stage C are not difficult to make out by a comparison of these two stages. To facilitate description I shall call the ventral arm of the U-shaped posterior cavity in stage B, as seen from the left side, the left ventral horn, and the right arm of the U, as seen from the ventral side, the right horn of the left posterior enterocœl. In passing from stage B to stage C the left ventral as well as the right horn of the left posterior enterocœl enlarges considerably, the former becoming more spacious, encroaching on the right side, and the latter, mostly extending backwards, so that the broad mesentery seen in stage B between these two horns is finally absorbed and leaves only an incomplete valve-like septum already described. The mesentery between the right and left posterior enterocœls is also absorbed nearly at the same time that the two horns just mentioned coalesce. Simultaneously with this fusion of the cavities on the ventral side, the secondary septum leading to the separation of the epigastric enterocœl is being formed. The fusion of the left and right posterior enterocœl, on the right side of the body must be rather abrupt, for in none of the series of sections that I have prepared of some stages intermediate to B and C have I observed the process agoing.

From the above description it follows that the line of fusion of the right and left posterior cavities must be the one indicated by a broken line *ab* in Fig. 8 (Pl. XIX).

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There is another portion of the body cavity which remains to be described in stage C; it is the "Centralblutgeflecht" of Ludwig ['82], the "dorsal organ" of Bury ['95], but which has clearly been shown by MacBride ['96] to be a portion of the enterocœl. The latter author calls it "oral cœlom"; but as this term has been applied to the left enterocœl in crinoids, I prefer to call it, from the position which it occupies in the star, "periœsophageal enterocœl." It arises as a bud from the dorso-dextral anterior end of the left posterior enterocœl in a stage between B and C; it is at first a solid rod of cells but afterwards acquires a lumen. In stage C (Figs. 19 & 22, Pl. XX) it is a tubular structure opening at one end into the left posterior enterocœl at the place above specified and ending blindly on the left side of the cardiac portion of the stomach at about $\frac{1}{3}$ the distance between the ventral and dorsal sides. It is irregularly curved, and proceeds from its origin at first dextrad then slightly anteriad and then sinistro-ventrad and posteriad; it therefore encircles at this stage about $\frac{1}{3}$ or perhaps $\frac{1}{4}$ of the stomach on the left side. It appears very conspicuous in cross sections at about the level of the pore-canal, in the mesenchyme lying between the left posterior enterocœl, the stomach, and the rudiment of the water-vascular system (Figs. 19 & 22).

In stage C the rudiment of the stone-canal is formed. It begins as a thickened elongated patch of the peritoneal epithelium on the dextro-dorsal wall of the left anterior enterocœl, contiguous to the inner opening of the pore-canal (Fig. 20, Pl. XXI). The thickened patch assumes the form of a furrow which subsequently closes into a canal, the closure commencing next the inner opening of the pore-canal, but leaving it untouched; so that if one were to look at the inner opening of the pore-canal from inside the anterior enterocœl, one would see it become somewhat elongated with the first formation of the rudiment of the stone canal, and then as the latter becomes closed there would be two openings,

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one that of the pore-canal, and the other that of the stone-canal, as represented in Fig. 25. These two openings would become more and more separated from each other, as the formation of the stone-canal proceeds. It is also evident from the above description that one side of the wall of the inner opening of the pore-canal is directly continued into the stone-canal.

From the above description of the first rudiment of the stone-canal, it is perfectly clear that it and the pore-canal are two quite distinct structures, there being no trace of a stone-canal in stage B. This fact has been generally recognized by students of asterid development *[Bury '87 & '98, MacBride '96, p. 351], and has received a just recognition in Lang's Lehrbuch; but as it is not so generally known I think it necessary to emphasize it here once more. No less an authority than Ludwig has remarked long ago ['82, p. 34), "Man hat sich daran gewöhnt den Rückenporus der Echinodermenlarven als die primäre Kommunikationsöffnung der Aussenwelt mit dem Wassergefässsysteme zu betrachten. Diese Auffassung halte ich nicht für ganz zutreffend. Man hat dabei einen Umstand nicht in Betracht gezogen, der eine grössere Beachtung verdiente—ich meine den Umstand, dass..... der Rückenporus anfänglich *niemals nur* mit dem Hydrocœl in Verbindung steht, sondern in einen Raum führt, der zugleich die linke Hälfte des Enterocœls oder das gesammte Enterocœl repräsentirt. Mir scheint es demnach den Thatsachen besser zu entsprechen, wenn man den Rückenporus in seiner primären Bedeutung als einen in das *Enterocœl* führenden Porus betrachtet, welcher erst sekundär in engere Beziehung zu dem von dem Enterocœl abgespaltenen Wassergefässsysteme tritt." This opinion has been fully confirmed by the researches of Bury and MacBride, and the results of my own observations are such an exact

* Field ['93, p. 120], however, does not seem to have been aware of the formation of the stone-canal independently of the pore-canal.

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confirmation of those of the latter that I would not have dwelt on this question so fully, were it not for the fact that mine were made on a species which passes through typical bipinnaria and brachiolaria stages, while the species studied by MacBride passes at most only through a highly modified form of the brachiolaria. We thus see that the behaviour of the pore-canal of asterids is exactly similar to that of the primary calyx pore (Kelchporus) of crinoids [Ludwig '80, Seeliger '92, p. 313] ; and the opinion of Bury ['87, p. 420] that the pore-canal has "existed in a very early stage in the history of Echinoderms, probably before the hydrocœl had arisen," is fully endorsed by what occurs in *A. pallida*.

In stage D the condition of the body cavities is essentially the same as in stage C. But the constriction of the left posterior enterocœl from the left anterior has been carried further ; and hand in hand with the rotation of the aboral disc the epigastric enterocœl has also shifted on towards the left side. These two, the aboral disc and the epigastric enterocœl, always go together, the latter lying directly under the former, as may be seen from Fig. 17, Pl. XX, which represents some of the frontal serial sections from stage D, placed in order from ventral to dorsal. The partial valve-like septum which we found in stage C between the coalesced left ventral and right horns of the left posterior cavity has completely disappeared, and there is but a single large cavity around the anus (Fig. 17 *a* & *b*). We can not, moreover, know where to draw the boundary line between the right and left posterior enterocœls, and it must be remarked that the posterior cavity next the hydrocœl, marked as the left posterior enterocœl, on the right side of the body in Fig. 17 *c* may just as well be a portion of the right posterior enterocœl, though I believe the right horn of the left posterior enterocœl has by this time extended so far dorsad as to include this region also. In Fig. 17 *d* one sees that the anterior and posterior

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enterocœls of the left side are separated only by a valve-like partial septum, and the complete separation of these two cavities is effected by the growth of this septum. In Fig. 17 *e* we see that the separation of the epigastric from the right posterior enterocœl is not yet quite thorough, the two being still connected by a two-layered cellular membrane like the sero-amniotic connection of vertebrates. There has also been a change in the outline form of the coalesced left and right posterior enterocœl as seen from the left side. In this stage (D) namely, the margin of the aboral disc presents five rounded protuberances which are to become the arms of the star ; and into each of these protuberances the coalesced left and right posterior cavity sends out a diverticulum (Fig. 9).

The epigastric enterocœl has grown somewhat more and has become more nearly circular, than in stage C, but it is still very flattened and is confined more to the dorsal side of the body, so that it does not appear in para-frontal sections passing through the most ventral portion of the body (Fig. 17 *a* & *b*).

Henceforth, *i.e.*, after the complete isolation of the epigastric enterocœl, I shall call the coalesced cavity consisting of the whole left posterior enterocœl and the part of the right posterior enterocœl that remains after the isolation, the "secondary left posterior enterocœl." This is in stage D still in open communication with the anterior enterocœl.

The periœsophageal enterocœl is nearly in the same tubular condition that it was in stage C. It is, however, now entirely or almost entirely cut off from the left posterior enterocœl, being in the latter case connected with it by a solid string of cells. It has also grown more towards the ventral side, so that it now encircles more than one-half the circumference of the cardiac portion of the stomach.

The stone-canal has been constantly growing away from the inner

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opening of the pore-canal, and makes an obtuse angle with the latter. The whole length of the canal is, however, inconsiderable.

In stage E the rudiments of the arms have become very marked, and with them also the diverticula of the secondary left posterior enterocœl, which enter them. The latter, which in the mean time, has been thoroughly separated from the anterior enterocœl, has extended to the right side on the ventral, as well as on the dorsal, region ; the whole forming, if we leave out of consideration the five diverticula of the arms, a thick horse-shoe shape, embracing about three-fourths of the stomach on the left side. We must also remember that at this stage the aboral disc is inclined to the sagittal plane at an angle of about 70° ; and consequently the plane of the horse-shoe formed by the secondary left posterior enterocœl makes nearly the same angle with the long axis of the larva. It must be remarked here that the secondary left posterior enterocœl is not yet separated from the anterior enterocœl of the right side, for from Fig. 12, it might perhaps be inferred that it is.

The epigastric enterocœl has become very nearly circular and has, at the same time, begun to send out diverticula into the arms, there being, in this stage however, only four diverticula (Fig. 12).

The periœsophageal enterocœl is nearly in the same condition in which it was in stage D ; only it has grown more around the stomach, encircling about half its circumference, its dorsal end remaining all the time nearly at the same point.

There is no change in the stone-canal worth noting, it having simply increased in length slightly. The plane containing the hydrocœlic pouches has, however, become inclined to the sagittal plane, but it makes with it a smaller angle than the aboral disc.

Stage F (Fig. 13) is a very interesting one: the aboral disc has assumed its definitive position, making a right angle with the sagittal plane, but the præoral portion of the body and especially the three

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brachiolar arms are still very distinct. In Fig. 18 is given a series of frontal sections of the specimen represented entire in Fig. 13*, beginning from the ventral side ; so that it is directly comparable with Fig. 17, which is taken from stage D.

Together with the aboral disc the epigastric enterocœl has assumed its definitive position and lies directly under the central portion of the former. It sends out diverticula into the arms of the star, which although distinct are yet short and blunt. In Fig. 18 *a*, *b*, and *f*, one sees these diverticula in transverse sections. The whole cavity is confined to the aboral side, and is even here encroached on more or less by the secondary left posterior enterocœl, as is clearly seen in Fig 18. This condition of the epigastric enterocœl persists, with a slight modification, to the adult stage.

The secondary left posterior enterocœl has been almost wholly cut off from the anterior enterocœl on the right side, by the formation of a septum from its walls at the boundary line between the anterior and posterior enterocœls. The coincidence in position of this imaginary boundary line and the septum just mentioned is so exact that I do not hesitate in affirming that this secondary separation takes place exactly at the place mentioned. The septum is in stage F still incomplete, as may be seen in Fig. 18 *c*, where the two cavities communicate with each other on the right side. The secondary left posterior enterocœl has now the form of a ring incomplete at one point, viz., on the dorsal median line, where the two portions are permanently separated from each other. It also sends out diverticula into the arms.

If the descriptions and their interpretations detailed above are correct, then the following equations must hold :

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* The shrinkage, which is considerable, is due solely to the usual treatment to which an object is subjected for imbedding in paraffin.

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Epigastric enterocœl=a portion of the right posterior enterocœl of larva.

Secondary posterior enterocœl=(left posterior enterocœl + right posterior enterocœl) of larva — epigastric enterocœl.

The anterior cœlom is still very spacious, though much less so than in any of the preceding stages, and the plane containing the hydrocœlic pouches makes an angle of about 70° with the sagittal plane, as may be seen from Fig. 18. Hand in hand with this change of the position of the plane, the curve formed by the hydrocœlic pouches has approached more nearly to a circle, and the first and fifth pouches are found on the right side of the body (Fig. 18 *a* & *f*). Neither pouch, however, has assumed its final position yet, as may be seen from Fig. 18, or from the following formula,

in which the Arabic numerals represent the hydrocœlic pouches and the Roman the arms of the star, while the members of the two series are placed in positions corresponding to what occurs in nature, as seen from the aboral side.

The pore-canal opens into the dorso-posterior corner of the anterior enterocœl, and the stone-canal makes with it a distinct angle,

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as may be inferred from a comparison of Fig. 18 *e* & *f*, between which there are three intervening sections (each = 6–7 μ). The calibre of the stone-canal is considerably smaller than that of the pore-canal.

The periœsophageal enterocœl encircles now fully one-half the circumference of the cardiac portion of the stomach. Its dorsal end has grown somewhat over to the right side of the body, and is now enlarged (Fig. 18 *e*).

In stages G to H important changes, the beginnings of which we have seen, are completed, and the several organs and cavities attain their definitive positions. The most salient of these changes are the formation of the water-vascular ring, the completion of the stone-canal and of the periœsophageal enterocœl, and the development of the perihæmal spaces and of the nervous system. And here at the outset I may state that I regard the two circular perihæmal spaces as of different origin; I shall therefore call the inner perihæmal ring "circular enterocœl." The justification for this name will be found in the following descriptions.

Hand in hand with the shrinking of the præoral lobe the anterior enterocœl becomes less and less spacious, and in Figs. 37–39, which are all taken from a stage intermediate to G and H, it is seen to have become very much flattened in an antero-posterior direction (*i. e.*, oral to aboral). In the interradius of the stone-canal, however, it is still somewhat spacious, and here it persists as the axial sinus, and receives the inner opening of the pore-canal and external opening of the stone-canal. The anterior enterocœl in thus becoming flattened has not been affected uniformly in all its parts, but its right and posterior portion lags behind the rest, as can be seen from Figs. 37, 38, and 39, the latter of which is pretty nearly the frontal section. When the flattening of the anterior enterocœl has

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reached its limit, the walls of the cavity are appressed against each other and form for a time a plate of cells, which subsequently disappears, probably by being drawn in into the formation of the wall of the axial sinus, which immediately afterwards undergoes a rapid enlargement with the growth of the star. Compare on this point Figs. 27 (*a*) and 39. The flattening and subsequent fusion of the walls of the anterior enterocœl leave two particular areas of a circular form untouched, and consequently two rings are formed, one the circular water-vascular canal and the other the circular enterocœl, or the inner perihæmal ring of authors. From the above description it is clear that the axial sinus is at last drawn into the primitive dorsal mesentery dividing the right from the left enterocœl in the larva.

The circular enterocœl is first formed on the left side (Figs. 27 & 39). This is in perfect accord with the fact above mentioned that the flattening of the anterior enterocœl is completed first on the same side. In the figures above referred to one sees the cavity in question entirely separated from the anterior enterocœl, but in some sections of the same series one can observe it still connected by an irregular string of cells with the plate of cells formed by the fusion of its walls. In Fig. 38, again, it is just in the process of being constricted off from the anterior enterocœl. The circular enterocœl is mostly situated nearer the centre of the disc than the water-vascular ring, and remains in permanent communication with the axial sinus in the madreporic interradius, where the constriction leading to its formation never takes place.

The reader has perhaps noticed in Fig. 18 (stage F) a posterior extension of the axial sinus marked *sin.' ax.'* In this stage it is almost completely cut off from the axial sinus proper, but can be seen in one or two sections to be connected with it by a few cells extending between their walls. It is that portion of the left anterior enterocœl which has been called "dorsal sac" by Bury, and about which we

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learned in an earlier stage. In the specimen from which Fig. 38 is taken it also lies close to the axial sinus on the posterior side. This cavity persists to the adult stage, as may be seen from Figs. 30, 41–45, & 47.

The water-vascular ring is formed precisely in the same way as the circular enterocœl, but in some places it remains connected by the plate of cells above mentioned with the anterior enterocœl longer than the latter (Figs. 27 & 39). In most parts the canal thus formed is very small, but in the five radii, *i.e.*, where it receives the radial water-vascular canal it is rather large (Fig. 39).

The formation of the two ring canals above described must not be considered as a simple mechanical effect of the formation of the metastome, as might be inferred from the descriptions of some preceding authors. As may be seen from the figures already referred to the two ring canals in question are complete some time before the formation of the metastome.

In a stage a little later than stage F the water-vascular pouches become 3-lobed, at the same time that the open curve formed by them approaches more nearly a circle. In stage G (Fig. 15) we see that the pouches are 5-lobed. The circle is not yet complete, but the plane containing the pouches is now almost perfectly paralled to the plane of the aboral disc. In stage H (Fig. 16) the circle is complete and the plane of the water-vascular system is perfectly parallel to that of the aboral disc. The lobes of the primary water-vascular pouches have become the ambulacral feet, with the exception of the terminal lobe which is destined to be the terminal tentacle. The disagreement between the numbers of the arms of the star and of the water-vascular pouches is at once apparent from Fig. 16—disagreement which has been recognized by previous students, and clearly pointed out in particular by Ludwig ['82].

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The secondary left posterior enterocœl encircles now the marginal portions of the stomach, forming a ring; but this ring is incomplete in the madreporic interradius, where the two arms remain permanently separated by the dorsal mesentery containing the axial sinus. Compare Figs. 28 & 37–39. Conforming with the gut in its general outline as seen in the antero-posterior direction, it sends out diverticula into the arms, in which it occupies not only the oral side of the gut but extends also on the aboral side, so that the mesentery separating it from the epigastric enterocœl lies always on the aboral side of the gut. The diverticula into the arms are in stages G and H still simple, but subsequently undergo a change of form corresponding to the change in form of the epigastric enterocœl, to the description of which we shall now pass on.

The central portion of the epigastric enterocœl presents nothing worth noting; we shall therefore concern ourselves only with its diverticula. These are, as already noted, at first simple, but hand in hand with the change of form which the hepatic cœca undergo, these diverticula also pass through similar changes. When the rudiment of each hepatic cœcum begins to divide into two branches to form the definitive double cœcum the diverticulum from the epigastric enterocœl also begins to be prolonged into two branches, both branches lying on the aboral side of the hepatic cœcum, and terminating at a short distance from the tip of the latter. The successive changes of form which the epigastric enterocœl undergoes can therefore be represented by cut 3. It need hardly be added that the diverticula of the secondary left posterior enterocœl undergo a corresponding change to the above.

The periœsophageal enterocœl has changed considerably compared with what it was in stage F. It has grown completely around the stomach, and its two ends have grown together and fused with

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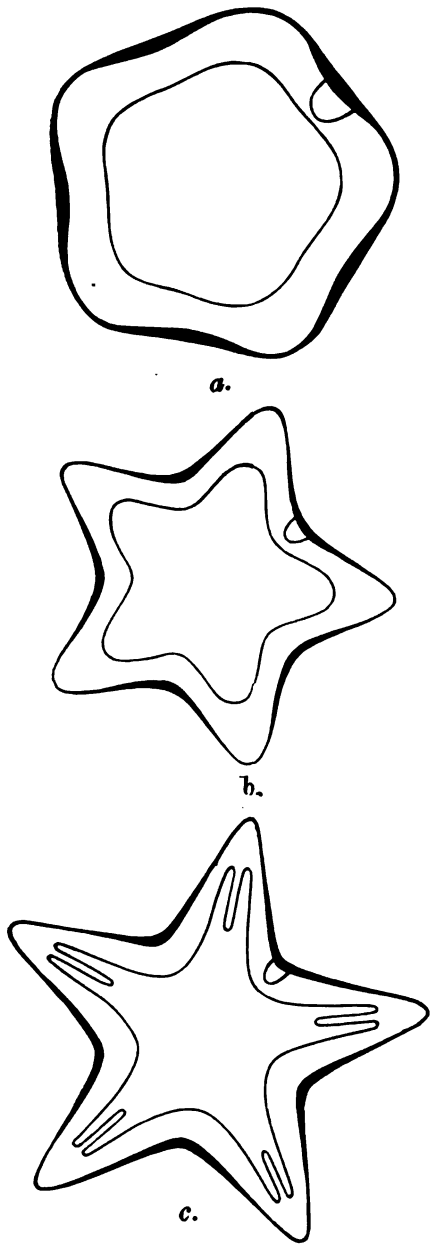

Cut 3.

each other. Its cavity has also enlarged considerably, so that it now forms a spacious circular ring around the permanent œsophagus (Figs. 27, 28, 36, 38, 39). In the madreporic interradius the periœsophageal enterocœl pushes in between the stomach and the axial sinus and thus confines the connection of the dorsal mesentery with the stomach to a very small area on the dorso-posterior corner of the gut (Fig. 38). In most of the figures representing the periœsophageal enterocœl in the condition above described (Figs. 27, 28, 36, 38, 39) there is a thick mesentery between it and the secondary left posterior enterocœl, but in Fig. 37, which is taken from the same series of sections as Fig. 38, this mesentery has disappeared, and the two cavities have become one. This coalescence of the two cavities takes place at all points, and in the complete star there is but one large cavity on the oral side of the gut, and we have then absolutely no means of recognizing the former existence of the periœsophageal enterocœl.*

* In my preliminary papers I stated that the mesentery in question seems to persist throughout life in the madreporic interradius. Further examination of more advanced stages has shown that the statement is erroneous.

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The permanent communication of the stone-canal with the axial sinus above described has been clearly recognized and stated by Bury ['95, p. 67] and MacBride ['93 & '95, p. 351], and Durham ['88] has also described such a direct connection in *Cribrella ocellata*. The latter, however, evidently regards the occurrence of such a communication as rather exceptional; and the statements of the majority of writers on asterid anatomy are against it. Hoffmann ['73, p. 16] among older writers has, indeed, asserted, from the result of injection, a connection of the blood system with external water by means of the axial sinus ; but this does not prove anything as to the communication of the latter with the stone-canal. Hamann, as well as Vogt and Jung, has also described a communication between the axial sinus and the surrounding cavities. According to Hamann ['85, p. 51] there is a direct connection between the enterocœl and the axial sinus at the point, "wo vom Chromatogenorgan Teile aus dem schlauchförmigen Kanal heraustreten, von welchen die Gefässe zum Mitteldarm abgehen, und diese Teile nicht von Fortsetzungen der Kanalwand umkleidet werden, sondern dieselbe einfach durchbrechen." Vogt and Jung ['88, p. 608, French edition p. 600] have described a direct connection between the axial sinus and the water-vascular system, and this connection takes place, according to these authors, by numerous fine openings. It must be remarked here that the method of injection is, to judge from a comparison of the statements of other-writers with the results of my own observations, in which I have traced the cavities through all stages of their development up to the complete star, very unreliable as to the question of communication between different cavities, unless indeed it be accompanied by exact microscopic observations of serial sections ; as a communication might take place by way of the meshes of the connective tissue where there is naturally none at all. In none of my sections have I observed such openings as are described by Vogt and Jung.

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The sum and pith of the statements of a large majority of previous writers on this point then is to the effect that there is no direct communication between the stone-canal and the axial sinus; and Ludwig, the acknowledged authority on echinoderms, sums up the question thus ['78, p. 104]: "Nicht eines der Porenkanälchen führt wo anders hin als in den Steinkanal (oder dessen nachher zu besprechende ampullenförmige Erweiterung); das gesammte Canalsystem der Madreporenplatte steht einzig und allein in Zusammenhang mit dem Steincanal, aber nicht mit dem schlauchförmigen Canal, noch auch mit irgend einem anderen Hohlraum." The direct connection of the stone-canal with the axial sinus is not even suspected. Cuénot is even more explicit on this point; he says ['87, p. 78], "quoi qu'en disent MM. Perrier, Carl Vogt et Jung, il n'y a jamais de perforations mettant en communication le canal du sable et les parties environnantes, pas plus à la base qu'au sommet."

It was with the hope of clearing up, if possible, this question that I undertook an examination in serial sections of the part concerned, and I may state at once that the result has been a complete confirmation of the statements of Durham, Bury, and MacBride. Figs. 30, 31 & 41-48 reproduce sections of the region concerned in the species I have examined, viz., *Asterias pallida*, *A. tenera*, *Solaster endeca*, *Asterina gibbosa*, *Cribrella sanguinolenta*, *Astropecten bispinosus*, and *Astrop. sp.* The dimensions of the specimens from which the sections were made are stated in the explanation of figures, and it will be seen that *Solaster endeca* and *Asterias tenera* are probably represented by young individuals. In all these figures the direct communication of the aboral end of the stone-canal and the axial sinus is very clearly seen and there is no question that it represents a normal state of things. In most species here represented this communication is single, but in the species of *Astropecten* from the Japanese coast represented by Fig. 48, there

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were two other connections besides the one shown in the figure on the left side and seven on the right side, making ten in all. In *Cribrella sanguinolenta* (Figs. 44 & 45) there is an ampulla directly underneath the madreporite on the right side of the median plane, connected with the axial sinus and receiving some of the pore-canals. In Fig. 45 one sees the connection of this ampulla with the axial sinus by means of a solid string of cells. The communication between the two cavities is, however, direct in one of the adjoining sections. The communication passage between the axial sinus and the stone-canal here described, is always on the right side of the median plane, and when single, runs through only a few sections, so that it is perhaps quite natural that the majority of observers have overlooked it.

We thus see that the axial sinus communicates with two cavities, on the aboral side with the stone-canal (as well as the pore-canal), and on the oral side with the circular enterocœl (Fig. 49). It may incidentally be remarked that some of the pore-canals open directly into the axial sinus, the case of *Cribrella* above mentioned being only one particular case among many others.

I shall now add a few notes on the histology of the epithelium of the hydro-enterocœl

The wall of the body-cavities of the bipinnaria consists of a single layer of exceedingly flattened cells whose boundaries can not be recognized by simple staining with hæmatoxylin. The protoplasm is finely granular and takes up the stain tolerably well. It secretes on the surface turned away from the cavity fine muscular fibrils. That these are really muscular I conclude from their optical properties and their behaviour towards stains, which are exactly similar to those of the muscular fibrils of the œsophagus. Most of these mesodermic muscle fibres run circularly around the body-cavity, *i.e.*, transversely to the antero-posterior axis of the body ; but there are also longitudinal fibres

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(Fig. 26 *b*). At the anterior extremity of the body-cavity these two sorts of fibres cross each other irregularly (Fig. 26 *a*). The presence of these fibres alters the whole aspect of the question whether the current passing through the pore-canal is excurrent or incurrent. The direction in which the cilia are turned in killed specimens proves nothing on this point ; but the presence of muscle fibres in the walls of the body-cavity makes it possible for external water to pass in as well as out. However, the effect of normal ciliary action I leave an open question, having no observations of my own to offer on this point.

The muscular fibrils above described not only persist, but are more strongly developed in the adult star where they are especially abundant on the aboral side.

The epithelium of the axial sinus presents a folding on the side next the stomach, into which the surrounding tissue extends—the whole forming the well known ovoid gland. This organ extends through nearly the whole length of the axial sinus and is attached to its wall a little to the left of the median line (Fig. 35).

The wall of the hydrocœl presents nothing specially different from that of the enterocœl in the earlier stages of metamorphosis, except that the five primary pouches have very thick walls. When the star is complete and the ring-canal has been formed, the walls of the whole hydrocœl system are much thicker than those of the enterocœl, and hence the two can easily be distinguished in sections. The pore-canal and the stone-canal have especially thickened walls. In Fig. 35 we see the rudiment of the fold in the wall of the stone-canal, which are to become more complicated in the adult star. As the star grows the difference just mentioned between the walls of the enterocœl and the hydrocœl becomes less and less pronounced, and in a star whose greater radius is 5 mm. it has entirely disappeared (Fig. 34). In a star whose radius is 2 mm. the musculature of the water-vascular system

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is well developed, but in a star, such as is represented in Fig. 16, in which the ambulacral feet have been developed I have not been able to detect any muscle fibres in the wall of the water-vascular system, although it can adhere to the substratum quite hard. The musculature of the hydrocœl is, like that of the enterocœl, developed on the external surface of its epithelium.

On the origin of the genital organs I have absolutely no observation to offer. In individuals whose radius was 5 mm. I was not able to find any trace of them.

IV. Cavities of Mesenchymatous Origin.

In this category I reckon the perihæmal spaces, *i.e.*, with the exception of the inner perihæmal ring-space which I have proved to be a true enterocœl, and the peribranchial cavities.

Perihæmal system.—My observations on the origin of this system have given a very different result from that of MacBride ['96]. It originates in a star which has just completed metamorphosis, as solid masses of mesenchymatous cells on the oral side of the radial water-vascular canals. They are situated in pairs on either side of an imaginary plane passing through the axis of each radial canal and cutting the disc at a right angle. They are also invariably present between the bases of the ambulacral feet, and the pair nearest the centre of the disc is situated at first between the water-vascular ring-canal and the first pair of ambulacral feet. Subsequently, however, it comes to lie directly on the oral side of the former. These solid masses of cells afterwards acquire lumen, and in this stage they are represented in Fig. 28. The cavities are at this stage entirely separate from one another, but they afterwards grow and fuse with each other, with this limitation that the mesentery lying between each pair persists, so that the spaces on one side of the radial plane com-

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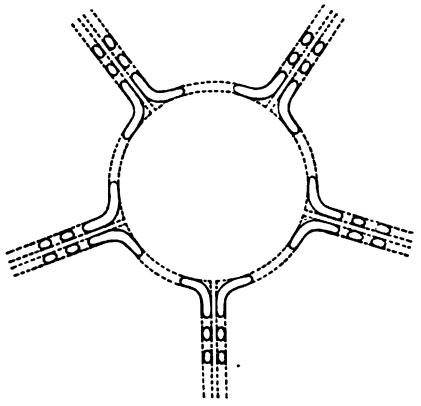
municate with each other but not with their fellows on the other side. The pair of perihæmal spaces lying nearest the centre of the disc grow around the water-vascular ring-canal in opposite directions away from the median mesentery, leaving this untouched but coalescing with the cavity of the next radius when the two have grown enough to meet each other, and thus form the perihæmal ring spaces. In the accompanying cut continuous lines represent these spaces before and dotted lines the condition after, fusion. In this way is brought about the condition of the perihæmal system that we see in the adult starfish, the central portion of which is represented diagrammatically, together with the circular enterocœl and the central portion of the water-vascular system, in Fig. 49.



Cut 4.

The correctness of my opinion above explained as to the origin of the perihæmal spaces is still more strengthened, if possible, by the condition that we meet with at the tip of the arms of a growing starfish. In Fig. 34 is represented a small portion next the terminal tentacle of a radial section of an arm of a young star, whose radius was 5 mm. Here we see that the perihæmal system consists of a number of entirely separate cavities, each lying between the roots of the tube-feet, although in the more central portion of the section the perihæmal space consists of a continuous cavity on either side of the radial plane. Hence we may infer that the peripheral portions of the perihæmal system of the young starfish grow by a repetition of just the same processes as those by which it was formed at its very beginning, viz., by the formation of separate mesenchymatous cavities and their subsequent fusion.

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Peribranchial cavities.— According to MacBride ['96, p. 381] the peribranchial spaces arise as diverticula from the enterocœl, although it must be remarked that he expresses some uncertainty connected with them. In my opinion these spaces are likewise of mesenchymatous origin, although it must be confessed that my observations on this question are not as complete as I could desire. In Fig. 33 I have reproduced a small portion of a section (transverse) of a lateral wall of one of the arms. It represents the earliest condition of the peribranchial spaces that has come under my notice. Here we see that there is a more or less distinct layer of cells next the peritoneal epithelium, similar to the latter. It is present at this stage (R=2 mm.) in all parts of the body. We see further that the peribranchial cavities are formed by the splitting of this cell layer. In the stage under consideration it is a ring-like space encircling the diverticulum of the enterocœl, which is to become the central cavity of the dermal branchia; and at the tip the space left by the ring is spanned by a thin cellular membrane, the direct continuation of the walls of the peribranchial cavity. This membrane must either represent the original condition of things or have been secondarily formed by the growth of the branchial diverticulum of the enterocœl, in consequence of which the walls of the central portion of the peribranchial cavity which was until then single and continuous, were applied to each other and have become fused. My observations are confessedly incomplete on this point.

As to the origin of the cell layer by the splitting of which the peribranchial cavities are formed, I have found no indication that it is derived from the peritoneal epithelium. It appears to me that it has been formed by a special arrangement of some cells of the connective tissue, as suggested on the lower side of Fig. 33.

October, 1897.

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PLATE XIX.

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Plate XIX.

ASTERIAS PALLIDA (Ag.).

All the figures in this plate are magnified 60 diameters. With the exception of Fig. 13, they were drawn from specimens mounted in balsam. The former was drawn in alcohol; hence the disproportion in size between it and the rest.

Stage B. 1. A fully developed bipinnaria seen from the left side.
„ 2. Ditto, from the left side, showing the body cavities.
„ 3. Ditto, from the ventral side.
„ 4. Ditto, from the right side.
Stage C. 5. A young brachiolaria seen from the left side.
„ 6. Ditto, from the left side, showing the body cavities.
„ 7. Ditto, from the ventral side.
„ 8. Ditto, from the right side.
Stage D. 9. A fully developed brachiolaria seen from the left side.
Stage E. 10. Brachiolaria undergoing metamorphosis into the star, from the left side.
„ 11. Ditto, from the left side, showing the body cavities.
„ 12. Ditto, from the right side. The anterior enterocœl has not been completely separated from the posterior enterocœl on the right side.
Stage F. 13. A still more advanced stage of metamorphosis, from the dorsal side.
Stage G. 14. Metamorphosis nearly completed, from the dorsal side.
„ 15. Ditto, from the anterior pole.
Stage H. 16. Metamorphosis just completed, from the anterior pole.

Posterior median
Posterior ventral
Posterior dorsal
Anterior dorsal
Anterior ventral
} tentacles are paired.

ABBREVIATIONS.

an. pro..... protoproct.
brach. d. ..... right brachiolaria arm.
„ med..... median „ „
„ s. ..... left „ „
can. aq..... pore canal.
can. aren..... stone canal.
cav. perihæm. ... perihæmal cavity.
ent'c'l. a. ..... anterior enterocœl.
„ cir. ..... circular „
„ epigas.... epigastric „
„ p. d..... right posterior enterocœl.
„ p. s. ..... left „ „
„ p'. s'. ..... secondary left posterior enterocœl.
„ perioes.... perioesophageal enterocœl.
gast. ..... stomach.
int..... intestine.
sin. ax. ..... axial sinus.
sin'. ax'. ..... closed portion of the axial sinus.
tent. dor. a..... anterior dorsal tentacle (= bipinnaria arm.)
„ „ p..... posterior dorsal tentacle.
„ med. a. ..... anterior median „
„ „ p. ..... posterior „ „
„ ven. a. ..... anterior ventral „
„ „ p. ..... posterior „ „
vas. aq. cir. ..... circular water-vascular canal.
„ „ rad..... radial „ „ „

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PLATE XX.

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Plate XX.

ASTERIAS PALLIDA (Ag.).

All the figures in this plate are magnified 60 diameters.

17. A series of frontal sections of a specimen in stage D. The sections are each 6-7 μ thick, and are represented as seen from the ventral side. *a* is the 16th, *b* the 37th, *c* the 50th, *d* the 66th, and *e* the 83rd section from the most ventral section, there being 120 sections in all.

18. A series of frontal sections of the specimen represented in Fig. 13. The shrinkage is due solely to the usual procedure of imbedding. The sections are each 6-7 μ thick and are represented as seen from the ventral side. There are 7 sections between *a* and *b*, 11 between *b* and *c*, 12 between *c* and *d*, 11 between *d* and *e*, and 5 between *e* and *f*.

ABBREVIATIONS.

an. pro.	protoproct.
brach. d.	right brachiolaria arm.
„ med.	median „ „
„ s.	left „ „
can. aq.	pore canal.
can. aren.	stone canal.
cav. perihæm.	perihæmal cavity.
ent'c'l. a.	anterior enterocœl.
„ cir.	circular „
„ epigas.	epigastric „
„ p. d.	right posterior enterocœl.
„ p. s.	left „ „
„ p'. s'.	secondary left posterior enterocœl.
„ perices.	pericœsophageal enterocœl.
gast.	stomach.
int.	intestine.
sin. ax.	axial sinus.
sin'. ax'.	closed portion of the axial sinus.
tent. dor. a.	anterior dorsal tentacle (= bipinnaria arm.)
„ „ p.	posterior „ „
„ med. a.	anterior median „
„ „ p.	posterior „ „
„ ven. a.	anterior ventral „
„ „ p.	posterior „ „
vas. aq. cir.	circular water-vascular canal.
„ „ rad.	radial „ „ „

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PLATE XXI.

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Plate XXI.

ASTERIAS PALLIDA (Ag.).

In the case of transverse sections they are represented as seen from the posterior pole.

19. Dorsal median portion of a cross section from stage C, through the level of the dorsal pore. ×395.
20. Ditto a little in front of the preceding. ×395.
21. Part of a transverse section from a stage somewhat prior to stage B (stage A), through the level of the inner opening of the pore-canal. ×250.
22. Right half of a transverse section from stage B, 8 sections (each=6-7 μ) behind the dorsal pore. ×250.
23. Dorsal portion of a transverse section from a stage a little prior to stage C, through the pore-canal. ×250.
24. A transverse section from stage C, through the posterior part of the hydrocœl. ×86.
25. Dorso-dextral portion of a transverse section from stage C, 15 sections (each =6-7 μ) behind the dorsal pore. ×390.
26. Diagrammatic representation of the separation of the inner openings of the pore-canal and stone-canal, as seen from within the left anterior enterocœl.
27. Muscular fibrils in the peritoneal epithelium of larva. *a* from the anterior extremity of the enterocœl, *b* from the region about the stomach. ×395.
28. A small portion of a radial section, next the metastome, from a stage a little prior to stage H. *a* plate of cells connecting the circular hydrocœl with the anterior enterocœl.
29. Portion of a radial section from a stage about the same as stage H. ×395.

ABBREVIATIONS.

an. pro..... protoproct.
brach. d. ..... right brachiolaria arm.
„ med..... median „ „
„ s. ..... left „ „
can. aq..... pore canal.
can. aren..... stone canal.
cav. perihæm. ... perihæmal cavity.
ent'c'l. a. ..... anterior enterocœl.
„ cir. ..... circular „
„ epigas.... epigastric „
„ p. d..... right posterior enterocœl.
„ p. s. ..... left „ „
„ p'. s'..... secondary left posterior enterocœl.
„ perioes.... perioesophageal.
gast. ..... stomach.
int. ..... intestine.
sin. ax. ..... axial sinus.
sin'. ax'. ..... closed portion of the axial sinus.
tent. dor. a..... anterior dorsal tentacle (= bipinnaria arm.)
„ „ p..... posterior dorsal tentacle.
„ med. a. ... anterior median „
„ „ p. ... posterior „ „
„ ven. a. ... anterior ventral „
„ „ p. ... posterior „ „
vas. aq. cir. ..... circular water-vascular cana
„ „ rad..... radial „ „

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PLATE XXII.

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Plate XXII.

ASTERIAS PALLIDA (Ag.).

30. Portion of a radial section through the madreporic interradius of a star whose R=2.5 mm. ×153.
31. Portion subjacent to the madreporite, from a radial section of a star whose R=5 mm. ×153.
32. A small portion of the section represented in fig. 40, to show the atrophy of the protoproct. Stage intermediate between F and G. ×395.
33. A small portion of a lateral wall of an arm of a star whose R=2.5 mm., to show the origin of the peribranchial cavity. ×395.
34. A small portion of a radial section of an arm next the terminal tentacle, of a star whose R=5 mm. ×395.
35. A portion of a horizontal section of the madreporic interradius of a star whose R=2 mm. ×153.
36. Central portion of a star in stage H; to show the formation of the metastome. ×395.

ABBREVIATIONS.

an. pro.	protoproct.
brach. d.	right brachiolaria arm.
„ med.	median „ „
„ s.	left „ „
can. aq.	pore canal.
can. aren.	stone canal.
cav. perihæm.	perihæmal cavity.
ent'c'l. a.	anterior enterocœl.
„ cir.	circular „
„ epigas.	epigastric „
„ p. d.	right posterior enterocœl.
„ p. s.	left „ „
„ p'. s'.	secondary left posterior enterocœl.
„ pericœs.	pericœsophageal enterocœl.
gast.	stomach.
int.	intestine.
sin. ax.	axial sinus.
sin'. ax'.	closed portion of the axial sinus.
tent. dor. a.	anterior dorsal tentacle (= bipinnaria arm.)
„ „ p	posterior „ „
„ med. a.	anterior median „
„ „ p.	posterior „ „
„ ven. a.	anterior ventral „
„ „ p.	posterior „ „
vas. aq. cir.	circular water-vascular canal.
„ „ rad.	radial „ „ „

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PLATE XXIII.

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Plate XXIII.

ASTERIAS PALLIDA (Ag.).

37. Parasagittal section of a star slightly prior to stage H; 7 sections (each=6-7 μ) on the right side of the next. ×153.

38. Sagittal section of ditto. ×153. The small cavity lying between the closed portion of the axial sinus (*sin'. ax'.*) and the pericesophageal enterocœl (on the left side of the figure) and connected with the latter by a solid string of cells is probably an accidental occurrence.

39. Frontal section of a star in stage H. ×135.

40. One of a series of sagittal sections of a transforming larva in a stage between F and G; to show the atrophy of the protoproct. Cf. Fig. 32. ×153.

41. Madreporic portion of a radial section of a star whose R=40 mm. ×60.

ASTROPECTEN BISPINOSUS, M. T.

42. Portion of a radial section directly under the madreporite; R=65 mm. ×50.

ASTERIAS TENERA, STIMPS.

43. Madreporic portion of a radial section of a specimen whose R=16 mm. ×60.

ABBREVIATIONS.

an. pro..... protoproct.
brach. d. ..... right brachiolaria arm.
„ med. ..... median „ „
„ s. ..... left „ „
can. aq..... pore canal.
can. aren..... stone canal.
cav. perihæm. ..... perihæmal cavity.
ent'c'l. a. ..... anterior enterocœl.
„ cir. ..... circular „
„ epigas..... epigastric „
„ p. d..... right posterior enterocœl.
„ p. s. ..... left „ „
„ p'. s'. ..... secondary left posterior enterocœl.
„ perices..... pericesophageal.
gast. ..... stomach.
int..... intestine.
sin. ax. ..... axial sinus.
sin'. ax'. ..... closed portion of the axial sinus.
tent. dor. a. ..... anterior dorsal tentacle (= bipinnaria arm.)
„ „ p. ..... posterior „ „
„ med. a. ..... anterior median „
„ „ p. ..... posterior „ „
„ ven. a. ..... anterior ventral „
„ „ p..... posterior „ „
vas. aq. cir. ..... circular water-vascular canal.
„ „ rad..... radial „ „ „

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PLATE XXIV.

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Plate XXIV.

CRIBRELLA SANGUINOLENTA, LÜTK.

44. Madreporic portion of a radial section of a specimen whose R=24 mm. ×60.
45. Ditto, 34 sections (each=10 μ) on the right side of the foregoing. ×60.

SOLASTER ENDECA (L.)

46. Madreporic portion of a radial section of a specimen whose R=6.5 mm. ×60.

ASTERINA GIBBOSA (Penn.).

47. Madreporic portion of a radial section of a specimen whose R=10 mm. ×60.

ASTROPECTEN SP.

48. Madreporic portion of a radial section of a specimen whose R=50 mm. ×54.

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49. Schematic representation of the central portion of the water-vascular and perihæmal systems, together with the circular enterocœl and the axial sinus. The radial water-vascular canals are wholly left out.

ABBREVIATIONS.

an. pro.	protoproct.
brach. d.	right brachiolaria arm.
„ med.	median „ „
„ s.	left „ „
can. aq.	pore canal.
can. aren.	stone canal.
cav. perihæm.	perihæmal cavity.
ent'c'l. a.	anterior enterocœl.
„ cir.	circular „
„ epigas.	epigastric „
„ p. d.	right posterior enterocœl.
„ p. s.	left „ „
„ p'. s'.	secondary left posterior enterocœl.
„ pericœs.	pericœsophageal enterocœl.
gast.	stomach.
int.	intestine.
sin. ax.	axial sinus.
sin'. ax'.	closed portion of the axial sinus.
tent. dor. a.	anterior dorsal tentacle (= bipinnaria arm.)
„ „ p.	posterior „ „
„ med. a.	anterior median „
„ „ p.	posterior „ „
„ ven. a.	anterior ventral „
„ „ p.	posterior „ „
vas. aq. cir.	circular water-vascular canal.
„ „ rad.	radial „ „ „

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明治三十一年六月廿二日印刷
明治三十一年六月廿五日發行

編纂兼發行者　東京帝國大學

東京市日本橋區兜町弐番地
東京印刷株式會社

印刷者　星野錫

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